AD NUMBER

ADB229442

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Specific Authority; 22 Sep 97. Other requests shall be referred to US Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.

AUTHORITY

USAMRMC ltr, 4 Dec 2002

AD_____

CONTRACT NUMBER DAMD17-97-C-7015

TITLE: Development and Validation of an Exogenous Metabolic Activation System for FETAX

PRINCIPAL INVESTIGATOR: Douglas J. Fort, Ph.D.

CONTRACTING ORGANIZATION: Stover and Associates, Inc.
Stillwater, Oklahoma 74075

REPORT DATE: June 1997

TYPE OF REPORT: Final, Phase I

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

9-22-97

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1997	Final, Phase I (12 Nov 96 - 11 May 97)

4. TITLE AND SUBTITLE

Development and Validation of an Exogenous Metabolic Activation System for FETAX

5. FUNDING NUMBERS

DAMD17-97-C-7015

6. AUTHOR(S)

Douglas J. Fort, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Stover and Associates, Inc.
Stillwater, Oklahoma 74075

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT 9-22-97

Distribution authorized to U.S. Government agencies only (specific authority). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) is a 96-hour, whole-embryo bioassay designed to detect potential developmental toxicants. The primary goal of this research was to improve the predictability and increase the overall utility of FETAX as a screen for developmental toxicants that pose a hazard to human health. The first specific aim was to explore the use of phenobarbital, ß-naphthoflavone, and isoniazid-induced rat liver microsomes as an exogenous metabolic activation system. This particular inducing system supplanted the presently-used Aroclor 1254 system which was recently proven to be somewhat unreliable. Post-isolation mixtures of these microsomes represented a broader spectrum of P-450 isozymes, and thus, bioactivated/inactivated a wider range of compounds. The second specific object was to develop and evaluate a standardized metabolic activation system test kit for FETAX which could be used by other FETAX users, including the U.S. Army. The findings from this research demonstrated that the post-isolation mixed microsomes were highly effective and that the test kits provided a reliable standardized means of routinely using the exogenous activation system with FETAX.

14. SUBJECT TERMS			15. NUMBER F PAGES
Developmental Toxicity	FETAX	P-450	202
Metabolic Activation System	*Xenopus*	Rat Liver	16. PRICE CODE
	Microsomes	Bioactivation	

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

DTF Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

DTF Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

6/5/97

PI - Signature Date

Table of Contents

Introduction

The vast majority of the epidemiological evidence acquired today suggests that most birth defects are the result of non-genetic environmental factors. Approximately 100,000-150,000 chemicals are currently available in the market place with 2,500 new chemicals released each year. Prior to their release into the market place and ultimately the environment, the safety of these compounds must be firmly established. The expense and time-consuming nature of conventional testing has warranted the development, validation, and eventual wide-spread use of alternative, non-mammalian model systems. FETAX (Frog Embryo Teratogenesis Assay - *Xenopus*) is a 4-day, non-mammalian, whole-embryo bioassay designed to evaluate potential teratogenic hazard by: 1) direct chemical/complex environmental mixture screening, and 2) use as a tool to evaluate toxicological mechanisms of action. FETAX has been extensively validated as a screening assay using both pure chemical compounds and complex environmental mixtures (wastewater effluent, sediments, and groundwater). Because *Xenopus* lack many metabolic enzyme systems including the mixed-function oxidase (MFO) system through the first 96-hours of development, an exogenous metabolic activation system (MAS) was developed (1) and evaluated (2-7) using Aroclor 1254-induced rat liver microsomes. However, the use of alternative, more advantageous inducing agents with the rat liver microsomes have only recently been explored with the FETAX assay.

Of approximately three million infants born alive each year, 13.3 per 1000 die within the first year (8). Approximately 2 to 3 percent of infants born alive have major congenital malformations recognized within the first year of life (9). When defects that only become apparent later in life are included, the frequency of major and minor malformations increases to about 16 percent (10). Approximately 7 percent of newborns are born prematurely (before the 37th week) and 7 percent of infants born at full term have low birth weights (2.5 kg or less) (11).

The NIOSH Registry of Toxic Substances (12) indicated that of some 38,000 chemicals in common industrial use, only 1,930 of these had been tested for teratogenecity with 580 demonstrating positive effects. In the Catalog of Teratogenic Agents complied by Thomas Shepard (13), over 600 chemical agents are presented with only 20 known to induce birth defects in humans. Obviously many gaps exist in our knowledge of how to predict which chemicals, among the million present in our environment or workplace, possess teratogenic potential; and the correlation between results in animal studies and effects in humans.

Developing an exogenous MAS for FETAX which is not dependent on Aroclor 1254 induction is imperative if FETAX is to be widely effective and universally accepted as a surrogate developmental toxicity screening assay. The use of Aroclor 1254 as an inducing agent presents several problems including: 1) inconsistent induction due to congener concentration variability from lot to lot, 2) isozyme repression (CYP2E1), 3) not readily available due to reduction in the use of PCBs, and 4) hazardous waste and human health hazard issues. Thus, developing post-isolation mixtures of rat liver microsomes induced by ß-naphthoflavone (CYP1A1 and 1A2), phenobarbital (CYP2A6/2B6), and isoniazid (CYP2E1) will help alleviate the problems associated with the use of Aroclor 1254 while providing a broad-spectrum of induced isozymes. The

previous mentioned isozymes represent the primary P-450 isozymes responsible for Phase I biotransformation of potential developmental toxicants. Additional inducing agents may be added to this repertoire as needed in the future.

By evaluating FETAX as a developmental toxicity screening system, the U.S. Army, scientific community, chemical and pharmaceutical producing industries, appropriate regulatory agencies, and ultimately the public will be provided with a versatile alternative, short-term prescreening assay and mechanistic model for the commonly used three segment test protocol. As opposed to many other advance technologies this bioassay can be developed, validated, and utilized quickly.

The *Xenopus* test system is a non-mammalian, alternative assay which offers some short and long-term solutions to current problems associated with developmental hazard evaluation. The problems are: increasing regulatory requirements, test cost, test duration, and the reduction in the use of mammals in research. Existing *in vivo* mammalian test systems are effective for the testing of drugs and cosmetics, but are too lengthy, expensive and technically difficult for evaluating mechanisms of action. In addition, the number of chemicals in production which require testing is increasing at a rate faster than is feasible to handle with *in vivo* test systems. These events have produced a climate which favors the use of alternative test systems for eventual refinement and possible reduction of *in vivo* assays. In a study conducted for NIH, the National Academy of Sciences recently concluded that the development and use of lower life form models for toxicity of testing should be emphasized. Tighter restrictions will limit the use of *in vivo* assays and increase the costs associated with their performance. The Office of Technology assessment for the United States Congress has recently completed an assessment of the use of animals in testing, research and education. Congressional options emanating from this study include support for the further development and use of *in vitro* test systems when appropriate. Thus, cost-effective, predictive developmentally relevant, tests are urgently needed to allow the rapid evaluation of developmental toxicants in the environment and to elucidate toxicological mechanisms of teratogenesis.

The ultimate goal of the proposed research is to protect humans from developmentally toxic chemicals, regardless of where exposure may take place, by either direct aversion or by increasing the understanding of toxicological mechanisms of teratogenesis. Knowledge of toxicological mechanisms of actions of commonly used drugs by physicians and pharmacists should increase confidence in the administrations of these drugs during pregnancy. In addition, this information will allow chemists and biologists to make educated, well-informed decisions on the development and manufacture of chemicals by understanding potential toxicological effects.

The specific goal of the proposed research was to improve the performance of FETAX by developing a more efficacious MAS using mixtures of ß-naphthoflavone-, phenobarbital, and isoniazid-induced rat live microsomes. Recent discussions with chemical and pharmaceutical manufacturing industries, as well as, the National Institute of Environmental Health Sciences (NIEHS), have indicated that these groups are interested in the FETAX system, but would not use or recommend the system on a large-scale basis unless the model could both screen for human developmental toxicants and elucidate mechanisms of action by including a standardized testing protocol with an effective MAS. Furthermore, the groups indicated that the screening assay must

be well developed and properly validated. Therefore, the development and proper validation of an exogenous MAS for FETAX must be performed before this assay can be successfully utilized.

The original work on developing a developmental toxicity screening assay using *Xenopus* embryos to detect environmental teratogens was performed in the laboratories of Greenhouse (14) and Dumont (15). Greenhouse used 48-hour exposures to military compounds (N-phenyl-α-naphthylamine and various hydrazines) to demonstrate toxic and teratogenic effects on developing embryos. Early studies in our laboratory have demonstrated that FETAX can be used with a variety of chemicals and complex mixtures. The endpoints included: LC50 (mortality), EC50 (malformation-teratogenesis), no observed effect concentration (NOEC), minimum concentration to inhibit growth (MCIG) [both length and developmental stage], motor behavior, pigmentation, and gross anatomy. A Teratogenic Index (TI), the ratio of the 96-h LC50/96-h EC50 (malformation) was developed and has been successfully used as a measure of the relative developmental hazard of a substance (1-7, 16-20). Thus, assessment of teratogenic potential has been based on TI values, embryo growth, and types and severity of induced malformations. Generally, TI values <1.5 have indicated low teratogenic potential, whereas higher values have signified an increase in the potential teratogenic hazard, as there was little or no separation between the concentrations that cause embryo malformation, but cause no embryolethal effects, and concentrations which were embryolethal. Greater TI values signified a larger separation between the two responses, and thus, a greater possibility of embryos being malformed in the absence of significant embryo lethality. Types and severity of induced malformations have also been considered, especially for compounds with TI values <1.5 which produce serious defects of major organ systems. Such compounds may still pose a serious threat, possibly as embryolethal agents. We are currently establishing a data base to assess the utility of the TI value in assessing teratogenic hazard. Test chemical exposures have been continuous for 96-hours. Mortality and stage of development were checked at hours 24, 48, 72, and 96, while the other endpoints were recorded only at hour 96. Test compounds renewal was performed daily throughout the tests. Data collection was simple, as all observations were made with a dissection microscope. The data collected using FETAX have been in harmony with the criteria for an *in vitro* teratogenesis screen suggested by Kimmel et al. (21). These criteria included: good concentration-response relationships, adequate number of embryos, and easily defined and quantified endpoints.

In addition, an American Society of Testing and Materials (ASTM) New Standard Guide for the Conduct of FETAX has been recently published (22). An "Atlas of Abnormalities" companion document to the ASTM guide has been produced in order to aid in the proper scoring of malformations (23).

FETAX Test Performance

Dumont (unpublished) has accumulated validation data on 45 compounds with nearly 85 percent correspondence to mammalian results. Sabourin (24) have completed testing of 32 compounds with 83% predictive accuracy. However, neither of these two investigators used the exogenous MAS developed for FETAX. With over 100 compounds tested, we have approached 95% predictive accuracy in our laboratory using the Aroclor 1254-induced exogenous MAS.

Correlation between laboratories has been encouraging, as well. For example, Courchesne and Bantle (25) found a teratogenic index for hydroxyurea of 4.3, whereas Sabourin recorded 4.5 for the same chemical. We have performed an extensive interlaboratory validation study with several laboratories across the United States (26-29). Results obtained were extremely encouraging and warranted further development and validation of a versatile, yet more robust MAS for FETAX.

Sabourin and Carlton (unpublished) determined that the same stock of diphenylhydantoin caused pericardial edema as the primary anomaly in both the cultured whole rat embryo and *Xenopus* embryos. Dumont et al. (30) similarly found that meclizine induced hydrocephalia in both frogs and mammals and that other teratogens produced similar abnormalities in both frogs and mammals (developmental mimicry). Courchesne and Bantle (25) reported that a number of genotoxic chemicals caused the same general types of malformations in both *Xenopus* and rodent embryos. Dawson et al. (16) have developed an artificial medium (FETAX solution) and have carried out a preliminary validation using five compounds ranging from a nonteratogen to a strong teratogen producing results corresponding to current mammalian literature. Fort et al. (1,3-7) have found that twenty-six known mammalian teratogens tested with FETAX produced similar malformations as observed in mammals.

Evaluation of Exogenous Metabolic Activation System

As mentioned in previous sections, the inability of young *Xenopus* embryos to metabolize xenobiotics prompted the development of an exogenous MAS. We developed the MAS by using Aroclor 1254-induced rat liver microsomes and an NADPH generating system co-cultured with the embryos. The extensively studied proteratogenic compound, cyclophosphamide (31), was used in the initial pilot study to assess the feasibility of the system (1). Results indicated that cyclophosphamide was bioactivated into an embryotoxic form using the exogenous MAS. Also, addition of the microsomes, antibiotics (used to combat bacterial contamination) and a NADPH generating system did not increase mortality and malformation rates above FETAX solution control values. Specifically, activation reduced the 96-hour LC50 of cyclophosphamide 5.7-fold from 8.0 to 1.4 mg/mL. The 96-hour EC50 (malformation) was reduced 15.5-fold from 6.2 to 0.4 mg/mL. The 96-hour TI was increased 2.7-fold upon activation from 1.3 to 3.5. The severity of the malformations were used in FETAX compared to uninduced rat liver microsomes and generating system (32).

Additional validation studies were performed with Aroclor 1254-induced microsomes co-cultured with *Xenopus* embryos (3). Rifampicin, 2-acetylaminofluorene, and benzo(α)pyrene were all bioactivated as expected, while cytochalasin D was inactivated. The inclusion of the MAS had no effect on the developmental toxicity of zinc as expected. Nicotine had a TI value of 325 when tested in FETAX without Aroclor 1254-induced rat liver microsomes (2). This value was reduced to 3.5 when the embryos were co-cultured with an Aroclor 1254-induced MAS. However, bioactivation did not always result in a change in the TI value. Bantle et al. (33) found that metabolic activation reduced the 96-hour LC50 and EC50 (malformation) of N-ethyl-N-nitrosourea by a factor of 73, but did not change the TI value.

Evaluating Toxicological Mechanisms of Action

Preliminary studies have demonstrated the utility and versatility of FETAX for evaluating the toxicological mechanisms of action of diphenylhydantoin (4). In the initial study, teratogenic potentials of diphenylhydantoin (DPH) and hydroxylated metabolites (HPPH) were evaluated with FETAX. *Xenopus* embryos were exposed to DPH and HPPH in two separate static-renewal experiments with and without the presence of an exogenous MAS for 96 hours. Two separate concentration-response tests were also conducted with DPH and HPPH with a MAS modulated by various MFO inhibitors [carbon monoxide (CO) (broad-spectrum cytochrome P-450), cimetidine (mainly cytochrome P-450), ellipticine (cytochrome P-4490], and an epoxide hydrolase inhibitor (cyclohexene oxide). Assessment of the potential teratogenic hazard was based on TI values, types and severity of malformations, and embryos growth endpoints. Addition of the intact MAS to DPH increased the 96-hour LC50 and EC50 (malformation) from approximately 74.5 and 32.4 mg/L to 126.4 and 62.4 mg/L, respectively. The TI was reduced 1.2-fold. Both *p*-HPPH and *m*-HPPH were much less developmentally toxic than DPH. CO and cimetidine inhibition of cytochrome P-450 maintained much of the developmentally toxicity of DPH, whereas ellipticine inhibition of cytochrome P-448 was much less effective in maintaining the developmental toxicity of DPH. Cyclohexene oxide inhibition of epoxide hydrolase markedly increased DPH-induced embryotoxicity decreasing the 96-hour LC50 from approximately 74.5 to 38.6 mg/L. These results suggested that unmetabolized DPH and an embryotoxic epoxide intermediate served as the teratogenic species in FETAX.

In a separate study, the developmental toxicity of isoniazid (INH) and the metabolites acetylhydrazide (AH) and isonicotinic acid (INA) were examined with FETAX (5). Late *Xenopus* blastulae were exposed to INH, AH, and INA for 96-hours in two separate static-renewal tests with and without the presence of three differently induced metabolic activation systems (MAS). The MAS consisted of uninduced, Aroclor 1254-induced, and INH-induced rat liver microsomes. Addition of the INH-induced MAS decreased the 96-hour LC50 of INH and AH approximately 1.6-fold and 7.0-fold, respectively. The 96-hour EC50 (malformation) of INH was virtually unaffected; however, the INH-MAS decreased the TI value nearly 1.8-fold. The 96-hour EC50 (malformation) of AH increased approximately 2.0-fold, decreasing the TI value 15.9-fold. INA yielded a TI value of 2.5. Neither the uninduced MAS nor the Aroclor 1254-induced MAS had an affect on any of the compounds tested and none of the MASs affected the developmental toxicity of INA. Results from this study suggested that MFO metabolism may have altered the developmental toxicity of INH *in vitro* by producing a more embryolethal, but less teratogenic metabolite(s) than INH or AH themselves.

Finally, potential mechanisms of acetaminophen-induced developmental toxicity were evaluated using FETAX (7). Early *Xenopus* embryos were exposed to acetaminophen for 96-hours in two definitive concentrations-response assays with and without an exogenous MAS. Two static-renewal tests with acetaminophen and the MAS treated with carbon monoxide, cimetidine, ellipticine, diethyl maleate, and supplemented with glutathione were also performed. Addition of the MAS decreased the 96-hour LC50 and EC50 (malformation) values of unactivated acetaminophen 3.9-fold and 7.1-fold, respectively. Addition of the carbon monoxide- and ellipticine-inhibited MAS, as well as, the glutathione-supplemented MAS decreased the

developmental toxicity of activated acetaminophen to levels near that of the unactivated parent compound. Cimetidine-inhibited MAS also reduced the developmental toxicity of acetaminophen, but not to the extent observed with the carbon monoxide- and ellipticine-inhibited, or glutathione-supplemented MAS Addition of the diethyl maleate-treated MAS substantially increased the developmental toxicity of acetaminophen. Results indicated that a highly reactive intermediate formed as the result of mixed function oxidase-mediated metabolism (possibly P-448) significantly increased the developmental toxicity of acetaminophen. Glutathione was also found to play a major role in intermediate detoxification *in vitro*.

In summary, the developmental toxicity of four test compounds (cyclophosphamide, coumarin, 2-acetylaminofluorene [2-AAF], and trichloroethylene) was evaluated using the traditional static-renewal method described in the ASTM Standard Guide for conducing FETAX (22) with minor modification. Differently-induced rat liver microsomes in the form of a test kit was co-cultured with FETAX in an identical fashion to that described in Experimental Methods Section. Results from these studies are provided in this report.

Body

Experimental Methods

Animal Care and Breeding

Xenopus adult care, breeding, and embryo collection were performed as described in the ASTM Standard Guide (22).

Metabolic Activation System(s)

The metabolic activation system consisted of post-isolation mixed sets of ß-naphthoflavone, phenobarbital, and isoniazid-induced rat liver microsomes. Several studies including some in our own laboratories have demonstrated that the use of Aroclor 1254-induced microsomes with compounds metabolized by Aroclor 1254-repressed isozymes (pyrazole/isoniazid-induced P-450) was counter-productive, thus isoniazid was also been selected as a P-450 inducing agent. In addition, several chemicals have been shown to be metabolized by Aroclor 1254-repressible isozymes including: trichloroethylene, acetone, ethanol, and N-nitrosodimethylamine. Since the use of commercial preparations of rat liver microsomes, including both S-9 and lypholyzed purified preparations, has been unsuccessful due to a combination of high residual Aroclor 1254 levels and lipid peroxidation-induced toxicity, we purified rat liver S-9 supernatant. Purification of the rat liver microsomes was performed by the method of Kitchin and Woods (34). The pelleted microsomes were resuspended in cold 0.05 M Tris-HCl buffer and the protein content was determined. For each experiment performed the same amount of P-450 activity was added to the dishes even though the amount of protein varied. P-450 activity inferred by the amount of formaldehyde generated from the N-demethylation of aminopyrine and N-nitrosodimethylamine by the method of Nash (35). After quantification, the microsomes were split into four aliquots and

frozen in liquid nitrogen for use in each succeeding day of the experiment. Freezing of microsomes was determined to be an acceptable procedure (36). Randomly selected aliquots of the differently induced microsome mixes were treated with CO immediately prior to co-culture with the *Xenopus* embryos.

Assay Protocol

All FETAX tests were conducted in accordance with the methods described in the ASTM Standard Guide (22) with the minor modifications described below. For test conducted with unactivated toxicant (no MAS), groups of 20 embryos were placed in 60 mm plastic Petri dishes with varying concentrations of the toxicant. The test compounds were dissolved in FETAX solution, a reconstituted water media designed for the culture of *Xenopus* larvae (16). One percent DMSO (v/v) was used as a co-solvent as needed (4). Four separate dishes of 20 embryos were exposed to FETAX Solution alone and designated FETAX Solution controls. For each definitive test, 12-18 concentrations were tested in duplicate. Tests conducted with the MAS and the inhibited MAS were also conducted in duplicate with 20 embryos per replicate concentration. Each activated treatment received 0.04 U/ml of APD activity (expressed as μM formaldehyde generated/minute), a NADPH generating system, and 100 U/mL penicillin--100 U/mL streptomycin. The NADPHH generating system will consisted of 3.5 mM glucose-6-phosphate, 0.31 IU glucose-6-phosphate dehydrogenase, 0.1 mM NADP, and 0.7 μM NADPH. Controls for the MAS, CO inhibited-MAS, cyclophosphamide [FETAX reference proteratogen] (2), and unactivated toxicant were also be conducted with each individual test.

One range test, one definitive unactivated test, and two separate definitive tests with and without the MAS were conducted with each compound in the same format utilized during Phase III of the recent interlaboratory study (ILS) conducted with FETAX (29). Although limited testing was performed with the Aroclor 1254-induced MAS, previous ILS work (26-29) suggested that the most successful approach for consistent interlaboratory testing was to perform testing in the following manner: 1) initial range test to estimate 96-hour LC50 and EC50 (malformation) values, 2) definitive unactivated test (no MAS) using sliding scale to select concentrations (29), and 3) two definitive concentration-response experience tests with and without the exogenous MAS using the predicted EC5 (malformation) and LC5, EC16 and LC16, EC50 and LC50, EC84 and LC84, and EC95 and LC95 from the definitive unactivated test. This approach has been highly effective in testing performed since the completion of the ILS program (26-29). The pH of all stock solutions was strictly maintained between 7.0-7.2 if possible. Embryos were cultured at 23±1°C throughout the test. All solutions will be removed every 24 hours of the 4-day test and fresh solutions will be added. Dead embryos were removed when solutions were changed. Following 96-hours of exposure, surviving embryos were fixed in 3.0% formalin, pH 7.0. The number of live-malformed embryos and the stage of development (37) were then be ascertained using a dissection microscope.

Data Analysis

Probit analysis (38) was used to determine the 96-hour median lethal concentration (96-hour LC50) the 96-hour EC50 (the concentration inducing gross terata in 50% of the surviving larvae) and their respective 95% fiducial intervals. Comparison of concentration-response data will be performed by the method of Sprague and Fogels (39). TI values (18) were determined for each test. Head to tail length will be measured as an indicator of embryo growth using Sigma Scan® digitizing software (Jandel Scientific, Corte Madera, CA) and a personal computer. Concentrations inducing growth inhibition (MCIG) were calculated using the Williams test (grouped, P=0.05).

Results and Discussion

Development of Exogenous Metabolic Activation System

Preliminary range finding results of tests with cyclophosphamide, coumarin, 2-AAF, and trichloroethylene,are provided in Table 1. Results from these unactivated definitive tests were used to generate the concentrations to be used in the evaluation of the differently induced MASs. As previously mentioned in the Experimental Methods section, tests with each of the four compounds with and without each of an Aroclor 1254-, phenobarbital-, ß-naphthoflavone-, and isoniazid-induced MAS were performed concurrently. In addition, the use of post-isolation mixes of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs were prepared and tested concurrently with the other treatments.

Results of MAS performance evaluation with cyclophosphamide is provided in Table 2. Unactivated cyclophosphamide or cyclophosphamide co-cultured with the isoniazid-induced MAS were not developmentally toxic at concentrations >4,000 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 1,500.0 and 590.0 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 1,700.0 and 610.0 mg/L, respectively. The ß-naphthoflavone-induced MAS less effectively bioactivated cyclophosphamide to 96-hour LC50 and EC50 (malformation) values of 3,090.0 and 1,810.0 mg/L, respectively. As previously mentioned, the isoniazid-induced MAS was ineffective in bioactivating cyclophosphamide. The post-isolation mixture of the phenobarbital-, ß-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating cyclophosphamide producing LC50 and EC50 (malformation) values of 1,340.0 and 400.0 mg/L, respectively. The mixed MAS also produced the greatest TI value of 3.4 and inhibited growth at comparable levels (100.0 mg/L) to the Aroclor 1254- and phenobarbital-induced MAS tests. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-induced, phenobarbital-induced, and mixed MASs were successful in bioactivating cyclophosphamide, whereas, the ß-naphthoflavone- and isoniazid-induced systems were less effective and ineffective in bioactivating cyclophosphamide, respectively. Bioactivated cyclophosphamide induced microencephaly, ophthalmic abnormalities, and severe skeletal kinking. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents, rabbits, and monkeys (1, 31). The results of the present study were not necessarily unexpected as cyclophosphamide has been shown to primarily bioactivated by

Table 1

Results of Unactivated Definitive FETAX Tests

Compound	LC50[1]	EC50[1,2]	TI[3]	MCIG[4]
Cyclophosphamide	6760.0 (6650.0 - 6880.0)	4830.0 (4710.0 - 4950.0)	1.4	6000.0
Trichloroethylene	490.0 (480.0 - 500.0)	51.0 (49.0 - 53.0)	9.6	400.0
Coumarin	168.7 (154.2 - 186.2)	59.8 (58.5 - 61.1)	2.8	100.0
2-Acetylaminofluorene	110.0 (105.0 - 115.0)	30.0 (25.0 - 35.0)	3.7	90.0

[1] Expressed as mg/L.
[2] Malformation.
[3] Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).
[4] Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

Table 2

Results of FETAX tests with Cyclophosphamide and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50[1]	EC50[1, 2]	TI[3]	MCIG[4]
1	None	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Aroclor 1254	1500.0 (1420.0-1580.0)	590.0 (510.0-680.0)	2.5	100.0
	Phenobarbital	1700.0 (1610.0-1810.0)	610.0 (530.0-700.0)	2.8	100.0
	β-Naphthoflavone	3090.0 (3000.0-3170.0)	1810.0 (1720.0-1910.0)	1.7	1000.0
	Isoniazid	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Mixed[5]	1340.0 (1260.0-1430.0)	400.0 (340.0-470.0)	3.4	100.0

(continued)

Results of FETAX tests with Cyclophosphamide and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

(continued)

Test No.	MAS	LC50[1]	EC50[1, 2]	TI[3]	MCIG[4]
2	None	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Aroclor 1254	1850.0 (1740.0-1980.0)	710.0 (620.0-800.0)	2.6	100.0
	Phenobarbital	1590.0 (1510.0-1670.0)	920.0 (870.0-970.0)	1.7	100.0
	β- Naphthoflavone	3130.0 (3000.0-3280.0)	1860.0 (1750.0-1970.0)	1.7	1000.0
	Isoniazid	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Mixed[5]	1690.0 (1580.0-1810.0)	720.0 (630.0-810.0)	2.4	100.0

[1] Expressed as mg/L.
[2] Malformation.
[3] Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).
[4] Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).
[5] Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

phenobarbital-inducible P-450, but with some cross specificity with aryl hydrocarbon hydroxylase isozymes (B-naphthoflavone-inducible P-450) (1,29).

Results of MAS performance evaluation with coumarin is provided in Table 3. Tests with unactivated coumarin produced 96-hour LC50 and EC50 (malformation), TI, and MCIG values of 129.4 mg/L, 49.0 mg/L, 2.6, and 50.0 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 68.9 and 25.9 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to a lesser extent to 93.3 and 51.5 mg/L, respectively. The ß-naphthoflavone-induced MAS effectively bioactivated coumarin to 96-hour LC50 and EC50 (malformation) values of 57.2 and 16.9 mg/L, respectively. The isoniazid-induced MAS was also less effective in bioactivating coumarin producing LC50 and EC50 (malformation) values of 98.0 and 46.1 mg/L. The post-isolation mixture of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating coumarin producing LC50 and EC50 (malformation) values of 37.3 and 17.3 mg/L, respectively. Each of the MASs did not appreciably alter the TI values The ß-naphthoflavone-induced MAS inhibited growth at 10.0 mg/L, whereas, the Aroclor 1254-induced and mixed MASs inhibited growth at 25.0 mg/L. The isoniazid- and phenobarbital-induced MASs tests did not appreciable alter growth compared to unactivated coumarin. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-induced, ß-naphthoflavone-induced, and mixed MASs were successful in bioactivating coumarin, whereas, the phenobarbital- and isoniazid-induced systems were less effective and ineffective in bioactivating coumarin, respectively. Bioactivated coumarin induced microophthalmia, craniofacial abnormalities, visceral edema, and mouth defects. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents and rabbits, however somewhat more controversial than cyclophosphamide (40). As with cyclophosphamide, the results of the present study were not necessarily unexpected as coumarin has been shown to primarily bioactivated by aryl hydrocarbon hydroxylase isozymes (B-naphthoflavone-inducible P-450) (41).

Results of MAS performance evaluation studies with 2-AAF are provided in Table 4. Tests with unactivated 2-AAF produced 96-hour LC50 and EC50 (malformation), TI, and MCIG values of 125.9 mg/L, 8.7 mg/L, 14.5, and 125.0.0 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 17.9 and 7.0 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to a lesser extent to 75.4 and 12.1 mg/L, respectively. The ß-naphthoflavone-induced MAS effectively bioactivated 2-AAF to 96-hour LC50 and EC50 (malformation) values of 17.4 and 7.2 mg/L, respectively. The isoniazid-induced MAS was also less effective in bioactivating 2-AAF producing LC50 and EC50 (malformation) values of 100.2 and 10.7 mg/L. The post-isolation mixture of the phenobarbital-, ß-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating 2-AAF producing LC50 and EC50 (malformation) values of 17.7 and 7.2 mg/L, respectively. Each of the MASs, with the exception of the isoniazid-induced MAS appreciably reduced the TI value of unactivated 2-AAF. The B-naphthoflavone-induced, Aroclor 1254-induced, and mixed MAS inhibited growth at 10.0 mg/L. The phenobarbital- and isoniazid-induced MAS tests inhibited growth at 50.0 and 100.0 mg/L, respectively. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-

Table 3

Results of FETAX tests with Coumarin and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50[1]	EC50[1, 2]	TI[3]	MCIG[4]
1	None	129.4 (127.2-131.6)	49.0 (48.1-55.8)	2.6	50.0
	Aroclor 1254	68.9 (62.6-75.8)	25.9 (23.1-29.1)	2.7	25.0
	Phenobarbital	93.3 (90.7-95.9)	51.5 (43.1-61.6)	1.8	50.0
	β-Naphthoflavone	57.2 (51.8-63.2)	16.9 (15.0-19.6)	3.4	10.0
	Isoniazid	98.0 (94.6-101.5)	46.1 (43.2-51.2)	2.1	50.0
	Mixed[5]	37.3 (32.4-43.0)	17.1 (15.0-19.6)	2.2	25.0

(continued)

Results of FETAX tests with Coumarin and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

(continued)

Test No.	MAS	LC50[1]	EC50[1, 2]	TI[3]	MCIG[4]
2	None	136.8 (130.6-143.2)	43.0 (38.0-47.1)	3.2	50.0
	Aroclor 1254	73.5 (68.4-79.0)	34.8 (31.0-39.1)	2.1	25.0
	Phenobarbital	94.8 (91.8-97.9)	53.2 (47.4-59.7)	1.8	50.0
	β- Naphthoflavone	64.0 (59.4-70.0)	19.7 (16.7-23.3)	3.3	10.0
	Isoniazid	106.6 (101.9-111.5)	48.4 (43.7-53.5)	2.2	50.0
	Mixed[5]	39.8 (35.1-45.1)	20.9 (18.5-23.4)	1.9	25.0

[1] Expressed as mg/L.
[2] Malformation.
[3] Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).
[4] Minimum Concentration to Inhibit Growth (Wiliams Test, P=0.05).
[5] Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

Table 4

Results of FETAX tests with 2-Acetylaminofluorene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50[1]	EC50[1, 2]	TI[3]	MCIG[4]
1	None	125.9 (120.8-131.8)	8.7 (6.4-8.0)	14.5	125.0
	Aroclor 1254	17.9 (17.4-18.4)	7.0 (6.1-8.0)	2.6	10.0
	Phenobarbital	75.4 (72.3-78.6)	12.1 (10.5-13.9)	6.2	50.0
	β-Naphthoflavone	17.4 (16.7-18.3)	7.2 (6.4-8.0)	2.4	10.0
	Isoniazid	100.2 (93.7-107.1)	10.7 (9.4-12.3)	9.4	100.0
	Mixed[5]	17.7 (15.9-19.7)	7.5 (6.5-8.6)	2.4	10.0

Table 4 (continued)

Results of FETAX tests with 2-Acetylaminofluorene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50[1]	EC50[1,2]	TI[3]	MCIG[4]
2	None	130.7 (126.5-135.0)	9.1 (8.2-10.1)	14.4	125.0
	Aroclor 1254	22.0 (20.4-23.6)	6.4 (5.5-7.4)	3.4	10.0
	Phenobarbital	78.7 (73.5-84.2)	28.1 (15.0-21.5)	4.3	50.0
	β-Naphthoflavone	21.0 (19.3-22.9)	6.7 (5.9-7.7)	3.1	10.0
	Isoniazid	90.8 (84.5-97.6)	14.2 (12.3-16.4)	6.4	100.0
	Mixed[5]	18.0 (15.8-20.5)	7.1 (6.4-7.9)	2.5	10.0

[1] Expressed as mg/L.
[2] Malformation.
[3] Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).
[4] Minimum Concentration to Inhibit Growth (William Test, P=0.05).
[5] Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

induced, ß-naphthoflavone-induced, and mixed MASs were successful in bioactivating 2-AAF, whereas, the phenobarbital- and isoniazid-induced systems were less effective and ineffective in bioactivating 2-AAF, respectively. Bioactivated 2-AAF induced pericardial edema, craniofacial mal-development, skeletal kinking, and microencephaly. These abnormalities were similar to those recorded in previous FETAX studies and similar to those identified in rodents and rabbits (3). As with cyclophosphamide, the results of the present study were not necessarily unexpected as 2-AAF has been shown to primarily bioactivated by aryl hydrocarbon hydroxylase isozymes (ß-naphthoflavone-inducible P-450) (42).

Results of MAS performance evaluation with trichloroethylene is provided in Table 5. Unactivated trichloroethylene induced 96-hour LC50, EC50 (malformation), TI, and MCIG values of 412.0 mg/L, 40.9 mg/L, 10.1, and 152.0 mg/L. In Test No. 1, the Aroclor 1254-induced MAS and the ß-naphthoflavone-induced MASs each did not appreciably alter the 96-hour LC50, EC50 (malformation), TI, and MCIG values. The phenobarbital-induced MAS only altered the 96-hour EC50 (malformation) by reducing it to 18.0 mg/L. The isoniazid-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 190.4 and 11.9 mg/L, respectively. The TI value was increased to 16.0. The post-isolation mixture of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating trichloroethylene producing LC50 and EC50 (malformation) values of 181.3 and 7.5 mg/L, respectively. The mixed MAS also produced the greatest TI value of 24.2 and inhibited growth at comparable levels (50.0 mg/L) to those observed with isoniazid-induced MAS tests. Results from Test No. 2 were similar to results from Test No. 1 in which each of the isoniazid-induced and mixed MASs were successful in bioactivating trichloroethylene, whereas, the ß-naphthoflavone-, Aroclor 1254-, and to a lesser extent the phenobarbital-induced systems were less effective in bioactivating trichloroethylene. Bioactivated trichloroethylene induced mal-developed guts, microencephaly, hydroencepahaly, hypognathia, microophthalmia, abnormal mouth development, and muscular kinking. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents, rabbits, and monkeys (6). The results of the present study were not necessarily unexpected as trichloroethylene has been shown to primarily bioactivated by isoniazid-inducible P-450 (43).

Raw data from the FETAX testing is provided as Appendix A.

Development of Metabolic Action System Test Kits

In order to facilitate the use of a simplistic metabolic activation system to use outside of our laboratory which would benefit the U.S. Army, and the scientific community, a MAS test kit was developed and evaluated in the aforementioned studies. The MAS test kit was developed and refined during Phase I studies and is proposed for use in the interlaboratory validation study which encompasses Phase II work. A model of the MAS test kit is included as Appendix B.

Table 5

Results of FETAX tests with Trichloroethylene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50[1]	EC50[1,2]	TI[3]	MCIG[4]
1	None	412.0 (370.4-458.3)	40.9 (35.4-42.3)	10.1	152.0
	Aroclor 1254	378.1 (339.5-421.4)	40.2 (34.6-46.6)	9.4	152.0
	Phenobarbital	412.1 (347.67-437.75)	18.0 (14.6-22.3)	22.9	152.0
	β-Naphthoflavone	390.1 (349.7-437.8)	39.7 (34.0-46.3)	9.8	152.0
	Isoniazid	190.4 (167.3-216.6)	11.9 (8.5-16.7)	16.0	50.0
	Mixed[5]	181.3 (158.8-206.9)	7.5 (6.5-8.7)	24.2	50.0

(continued)

Table 5 (continued)

Results of FETAX tests with Trichloroethylene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50[1]	EC50[1,2]	TI[3]	MCIG[4]
2	None	426.0 (384.5-472.9)	36.2 (30.8-42.6)	11.8	152.0
	Aroclor 1254	383.5 (341.6-430.5)	37.3 (32.0-43.4)	10.3	152.0
	Phenobarbital	410.8 (367.17-459.6)	19.5 (16.4-23.1)	21.1	97.0
	β-Naphthoflavone	388.9 (344.6-438.9)	40.2 (34.2-47.3)	9.7	152.0
	Isoniazid	175.3 (153.4-200.2)	11.5 (8.7-15.2)	15.2	50.0
	Mixed[5]	169.1 (147.8-193.5)	7.6 (6.4-9.1)	22.3	50.0

[1] Expressed as mg/L.
[2] Malformation.
[3] Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).
[4] Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).
[5] Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

Conclusions

Results from these studies suggested that the Aroclor 1254-induced MAS could effectively be replaced by a mixed lot of phenobarbital-, ß-naphthoflavone-, and isoniazid-induced rat liver microsomes. In addition, the use of a MAS test kit is a practicable means of facilitating external use of the system outside of our laboratory, particularly by the U.S. Army. Overall, the results from these studies suggested that continued validation of an exogenous metabolic activation system for FETAX is warranted in Phase II SBIR Studies to increase the overall predictability of the assay, and thus acceptance by the scientific and regulatory communities.

References

1. Fort, D.J., Dawson, D.A., and J.A. Bantle. Development of a metabolic activation system for the frog embryo teratogenesis assay: *Xenopus* (FETAX). Teratogen. Carcinogen. Mutagen. 8:251-263, 1988.
2. Dawson, D.A., Fort, D.J., Smith, G.L., Newell, D.L., and J.A. Bantle. Comparative evaluation of the developmental toxicity of nicotine and cotinine with FETAX. Teratogen. Carcinogen. Mutagen. 8:329-388, 1988.
3. Fort, D.J., James, B.L., and J.A. Bantle. Evaluation of the developmental toxicity of five compounds with the frog embryo teratogenesis assay: *Xenopus* (FETAX). J. Appl. Toxicol. 9:377-389, 1989.
4. Fort, D.J. and J.A. Bantle. Use of frog embryo teratogenesis assay-*Xenopus* (FETAX) and an exogenous metabolic activation system to evaluate the developmental toxicity of diphenylhydantoin. Fund. Appl. Toxicol. 14: 720-733, 1990.
5. Fort, D.J. and J.A. Bantle. Analysis of the mechanism of isoniazid-induced developmental toxicity with frog embryo teratogenesis assay - *Xenopus* (FETAX). Teratogen. Carcinogen. Mutagen. 10:463-476, 1990.
6. Fort, D.J., Rayburn, J.R., DeYoung, D.J., and J.A. Bantle. Assessing the efficacy of an Aroclor 1254-induced exogenous metabolic activation system for FETAX. Drug Chem. Toxicol. 14:143-161, 1991.
7. Fort, D.J., Rayburn, J.R., and J.A. Bantle. Mechanism of acetaminophen-induced developmental toxicity *in vitro*. Drug Chem. Toxicol. 15:329-350, 1992.
8. National Center for Health Statistic (NCHS). Births, marriages, divorces, and deaths for 1979. Monthly Vital Statistics Report. U.S. Department of Health, Education, and Welfare, 1980.
9. National Foundation/March of Dimes. Facts 1980. The Foundation, New York, 1981a.
10. Chung, C.S. and N.C. Myianthopoulos. Factors affecting risk of congenital malformations. The National Foundation March of Dimes Original Articles Series, Vol. XI, No. 10, 1975.
11. U.S. Department of Health, Education and Welfare (USDHEW). The Women and their Pregnancies. The Collaborative Perinatal Study of the National Institute of Neurological Diseases and Strokes. DHEW Publication No. (NIH) 73-379, 1972.
12. National Institute of Occupational Safety and Health (NIOSH). Registry of Toxic Substances, 1977.
13. Shepard, T.H., Fantel, A.G., Mirkes, P.E., Greenaway, J.C., Faustman-Watts, E., Campbell, M., and M.R. Jachau. Teratology testing: I. Development and status of short-term prescreens. II. Biotransformation of teratogens as studies in whole embryo culture. In: Developmental Pharmacology. Eds. S.M. MacLeod, A.B. Okey, and S.P. Spielberg. Raven Press, New York, pp. 147-164, 1983.
14. Greenhouse, G.. The evaluation of toxic effect of chemicals in fresh water by using frog embryos and larvae. Environ. Contam. Toxicol. 20:93-95, 1978.
15. Dumont, J.N., Schultz, T.W., Buchanan, M., and G. Kao. Frog embryo teratogenesis assay: *Xenopus* (FETAX) A short-term assay applicable to complex environmental mixtures. In Waters, Sandhu, Lewtas, Claxton, Chernoff, and Nesnow Eds.: "Short-

Term Bioassays in the Analysis of Complex Environmental Mixtures III." New York: Plenum Publishing, pp. 393-405, 1983.

16. Dawson, D.A. and J.A. Bantle. Development of a reconstituted water medium and initial validation of FETAX. J. Appl. Toxicol. 7:237-244, 1987.
17. Bantle, J.A., Dawson, D.A., and D.J. Fort. Identification of developmental toxicants using the frog embryo teratogenesis assay-*Xenopus* (FETAX). Hydrobiologia 188/189:577-585, 1989.
18. Dawson, D.A., Fort, D.J., Newell, D.L., and J.A. Bantle. Developmental toxicity testing with FETAX: evaluation of five validation compounds. Drug Chem. Toxicol. 12:67-76, 12:67-76, 1989.
19. Bantle, J.A., Fort, D.J., Rayburn J.R., DeYoung D.J., and S.J. Bush. Further Validation of FETAX: Evaluation of the development toxicity of five known mammalian teratogens and non-teratogens. Drug Chem. Toxicol. 13:267-283, 1990.
20. DeYoung, D.J., Bantle, J.A., and D.J. Fort. Assessment of the Developmental Toxicity of Ascorbic Acid, Sodium Selenate, Coumarin, Serotonin and 13-Cis retinoic acid using FETAX. Drug Chem. Toxicol. 14:127-143, 1991.
21. Kimmel, G.L., Smith, K., Kochar, D.M., and R.M. Pratt. Overview of *in vitro* teratogenicity testing: aspects of validation and application to screening. Teratogen. Carcinogen. Mutagen. 2:211-229, 1982.
22. Standard Guide for Conducting the Frog Embryo Teratogenesis Assay - *Xenopus* (FETAX). American Society for Testing and Materials (ASTM), E 1439-91, 1991.
23. Bantle, J.A., Dumont, J.N., Finch, R.A., and G. Linder. Atlas of Abnormalities: A Guide for the Performance of FETAX. Oklahoma State University Press, 1991.
24. Sabourin, T.D. and R.T. Faulk. Comparative evaluation of a short-term test for developmental effects using frog embryos. Branbury Report 26: Developmental Toxicology: Mechanisms and Risk. pp. 203-223, 1987.
25. Courchesne, C.L. and J.A. Bantle. Analysis of the activity of DNA, RNA, and protein synthesis inhibitors on *Xenopus* embryo development. Teratogen. Carcinogen. Mutagen. 5:177-193, 1985.
26. Bantle, J.A. Burton, D.T., Dawson, D.A., Dumont, J.N., Finch, R.A., Fort, D.J., Linder, G., Rayburn, J.R., Buchwalter, D., Maurice, M.A., and S.D. Turley. Initial interlaboratory validation study of FETAX: Phase I testing. J. Appl. Toxicol. 14(3): 213-223, 1994.
27. Bantle, J.A. Burton, D.T., Dawson, D.A., Dumont, J.N., Finch, R.A., Fort, D.J., Linder, G., Rayburn, J.R., Buchwalter, D., Gaudet-Hull, A.M., Maurice, M.A., and S.D. Turley. FETAX interlaboratory validation study: Phase II testing. Environ. Toxicol. Chem. 13(10):1629-1637, 1994.
28. Bantle, J.A. Burton, D.T., Dawson, D.A., Dumont, J.N., Finch, R.A., Fort, D.J., Linder, G., Rayburn, J.R., Buchwalter, D., Gaudet-Hull, A.M., Maurice, M.A., and S.D. Turley. FETAX interlaboratory validation study: Phase III testing. J. Appl. Toxicol., in press.
29. Fort, D.J., Stover, E.L., Bantle, J.A., Rayburn, J.R., Hull, M.A., Finch, R.A., Maurice, M.A., Burton, D.T., Turley, S.D., Dawson, D.A., Linder, G., Buchwalter, D., Dumont, J.N., and A.M. Gaudet-Hull. Interlaboratory validation of an exogenous metabolic activation system for frog embryo teratogenesis assay - *Xenopus* (FETAX). Drug Chem. Toxicol., in press.

30. Dumont, J.N., Schultz, T.W., and R.G. Epler. The response of the FETAX model to mammalian teratogens. Teratology 27:39a, 1983.
31. Mirkes, P.E. Cyclophosphamide teratogenesis: A review. Teratogen. Carcinogen. Mutagen. 5:75-88, 1985.
32. Bantle, J.A., and Dawson, D.A., Uninduced rat liver microsomes as an *in vitro* metabolic activation system for the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). In: Proceedings of the 10th Aquatic Toxicology and Hazard Assessment: 10th Volume, ASTM 971. Eds. W.J. Adams, G.A. Chapman and W.G. Landis. American Society for Testing and Materials, Philadelphia, pp. 316-326, 1987.
33. Bantle, J.A., Fort, D.J., and D.A. Dawson. Bridging the gap from short-term teratogenesis assays to human health hazard assessment by understanding common modes of teratogenic action. Proceedings of the 12th Aquatic Toxicology Symposium, ASTM 1027, American Society of Testing and Materials Philadelphia, pp. 46-58, 1989.
34. Kitchin, K.T., Schmid, B.P., and M.K. Sanyal. Teratogenicity of cyclophosphamide in a coupled microsomal activation/embryo culture system. Biochem. Pharmacol. 30:59-64, 1981.
35. Nash, T. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochemistry 55:412-416, 1955.
36. Dent, J.G., Schnell, S., Graichen, M.E., Allen, P., Abernathy, D., and D.B. Couch. Stability of activating systems for *in vitro* mutagenesis assays: Enzyme activity and activating ability following long-term storage and -85°C. Environ. Mutagen. 3:167-179, 1981.
37. Nieuwkoop, P.D. and J. Faber. Normal Table of *Xenopus laevis* (Daudin, Ed.), North Holland, Amsterdam, p. 52, 1975.
38. Tallarida, R.J. and R.B. Murray. Manual of Pharmacological Calculation with Computer Programs, Springer-Verlag, New York, p. 83, 1980.
39. Sprague, J.B. and A. Fogels (1977). Watch the y in bioassay. In: Environmental Protection Service Technical Report, No. EPS-5-AR-77-1, Nova Scotia, Canada, pp. 107-118, 1977.
40. DeYoung, D.L., Bantle, J.A., and D.J. Fort. Assessment of the developmental toxicity of ascorbic acid, sodiumselenate, coumarin, serotonin, and 13-cis retinoic acid using FETAX. Drug Chem. Toxicol., 14:127-41,1991.
41. Fort, D.J., Stover, E.L., Propst, T., Hull, M.A., and J.A. Bantle. Evaluation of the developmental toxicities of coumarin, 4-hydroxycoumarin, and 7-hydroxycoumarin using FETAX. Drug Chem. Toxicol., in press.
42. Propst, T.L., Fort, D.J., Stover, E.L., Schrock, B. and J.A. Bantle. Evaluation of the development toxicity of benzo[α]pyrene and 2-acetylaminofluorene using *Xenopus*: modes of biotransformation. Drug Chem. Toxicol., in press.
43. Fort, D.J., Stover, E.L., Rayburn, J.R. Hull, M. and J.A. Bantle. Evaluation of the developmental toxicity of trichloroethylene and detoxificatin metabolities using *Xenopus*. Teratogen. Carcinogen. Mutagen., 13:35-45, 1993.

Bibliography

Publications

1. D.J. Fort, T.L. Propst, B.C. Faulkner, E.L. Stover, J.A. Bantle, and R.A. Finch. Utilization of a broad-spectrum of induced rat liver microsomes is an effective metabolic activation system for FETAX, Drug Chem. Toxicol., in preparation.

2. D.J. Fort, T.L. Propst, B.C. Faulkner, E.L. Stover, J.A. Bantle, and R.A. Finch. Utilizing differently induced and inhibited rat liver microsomes to evaluate modes of biotransformation and detoxification, J. Appl. Toxicol., in preparation.

Abstracts and Presentations

1. D.J. Fort. Applications of FETAX, NIEHS FETAX Workshop, RTP, NC, April 1997.

2. D.J. Fort, T. Propst, amd E.L. Stover. Evaluating Mechanisms of Action of Development Toxicants Using FETAX as a Model, Society of Toxicology, Cincinnati, OH, March 1997.

3. D.J. Fort, T. Propst, and E.L.Stover. Evaluating Biotransformation of Development Toxicants Using FETAX as a Model, Teratology Society, Palm Beach, FL, June 1997.

4. D.J. Fort, E.L. Stover, T. Propst, B. Faulkner, J.A. Bantle, and R. Finch. Validation of a Whole-Embryo Limb Development Assay Using *Xenopus laevis*. Society of Environment Toxicology and Chemistry (SETAC), San Francisco, CA, November 1997.

Project Personnel

Enos L. Stover, Ph.D., P.E., DEE
Douglas J. Fort, Ph.D.
Kendall W. King, P.E.
John Scott
Susan Schaefer
Pat Lee
Timothy Propst
Brian Faulkner
Curtis Mirkes
Scott Shannon
Tommy Wynn
John Delphon
Micheal Copenhaver

Appendix A

Raw Data

FETAX SUMMARY SHEET

Test No. 1

Test Material: Coumarin	Investigator: Font
Source: US Army SBLB	Lab: SA
CAS No. / Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent / Conc.	Test Units (i.e., mg/ml): mg/L

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL: No. Dead or Malformed / Total Number X 100 = %	MORTALITY RECORD	MALFORMATION RECORD
	0 : 80 X 100 = ___ %	0 : 80 X 100 = 0 %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length ___ mm	Solvent Control Length ___ mm	
Minimum Concentration to Inhibit Growth (MCIG)		

UNMutagenic/BNF/INH/MIX/Arador/PB

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		93.32	51.49
LC_{50} 129.36/57.19/98.0/37.33/68.87		EC_{50} 49.01/16.94/46.13/17.14/25.89/	
95% Confidence limits 127.19-131.56/51.75-63.20 94.64-101.49/32.43-42.97 62.61-75.15		95% Confidence Limits 48.15-51.70/14.97-19.62/23.07-29.05/ 48.08-55.71/14.89-19.27/	
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})			43.06-61.58

90.72-95.92

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2500 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX MORTALITY DATA

Investigator Fort	**Test Material** Coumarin
Date	**Test Number** 1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
FX							0	0		
FX							0	0		
MAS										
5										
20							0	0	0	
27							0	0	0	
60							0	0	0	
86							0	0	0	
100							0	0	0	
115							0	0	0	
150							1	2	3	
200							20	20	40	
5							20	20	40	
20							0	0	0	
27							0	0	0	
60							3	2	5	
↓							5	6	11	
5							20	20	40	
20							0	0	0	
27							0	0	0	
60							0	0	0	
86							0	0	0	
100							3	2	5	
115							10	6	16	
↓							20	20	40	

Unext.

BNF

INH

FETAX MORTALITY DATA

Investigator	**Test Material** Coumarin
Date	**Test Number** 1

	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
MIX	5							0	0	0	
	20							3	1	4	
	27							7	5	12	
	60							11	17	28	
	↓							20	20	40	
Aroclor	5							0	0	0	
	20							0	0	0	
	27							1	2	3	
	60							5	3	8	
	86							10	12	22	
	100							20	20	40	
	115							20	20	40	
PB	5							0	0	0	
	20							↓	↓	0	
	27									0	
	60									0	
	86							2	1	3	
	100							20	13	33	
	115							20	20	40	

FETAX MALFORMATION DATA

Investigator: Fort	Test Material: Coumarin
Date:	Test Number:

MALFORMATION	CONCENTRATION																									TOTAL
						UN						BNF				INH						MIX				
	Fx	Fx	5	20	27	60	86	100	115	5	20	27	60	5	20	27	60	86	10	115	5	20	27	60		
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	2	10	24	40	40	37	0	15	35	29	0	0	8	23	35	24	–	0	13	16	12		
Total No.	40	40	40				→	40	37	40	40	35	29	40			→	35	24	–	40	36	28	12		

Comments:

FETAX MALFORMATION DATA

Investigator	Test Material
Date	Test Number

Aroclor PB

MALFORMATION	CONCENTRATION																											TOTAL
	5	20	27	60	86	27	60	86	100																			
Severe																												
Stunted																												
Gut																												
Edema																												
Multiple																												
Cardiac																												
Abdominal																												
Facial																												
Cephalic																												
Optic																												
Tail																												
Notochord																												
Fin																												
Face																												
Eye																												
Brain																												
Hemorrhage																												
Cardiac																												
Blisters																												
Other-specify																												
No. Malformed	0	4	26	32	18	8	22	29	7																			
Total No.	40	40	40	32	18	40	40	37	7																			

Comments:

5 20

0/40 0/40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN UNACT.
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	100.00	115.00	150.00	.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	0	0	0	0	
MORTALITIES:	0	3	40	0	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 129.36
VAR OF LN OF EST. : .71284D-04
95% LOWER CONFIDENCE: 127.19
95% UPPER CONFIDENCE: 131.56

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	5	11	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 57.19
VAR OF LN OF EST. : .24975D-02
95% LOWER CONFIDENCE: 51.75
95% UPPER CONFIDENCE: 63.20

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	5	16	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 98.00
VAR OF LN OF EST. : .30504D-03
95% LOWER CONFIDENCE: 94.64
95% UPPER CONFIDENCE: 101.49

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	4	12	28	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 37.33
VAR OF LN OF EST. : .49454D-02
95% LOWER CONFIDENCE: 32.43
95% UPPER CONFIDENCE: 42.97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	8	22	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 68.87
VAR OF LN OF EST. : .22691D-02
95% LOWER CONFIDENCE: 62.61
95% UPPER CONFIDENCE: 75.75

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	33	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 93.32
VAR OF LN OF EST. : .18934D-03
95% LOWER CONFIDENCE: 90.79
95% UPPER CONFIDENCE: 95.92

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	2	10	14	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 49.01
VAR OF LN OF EST. : .41671D-02
95% LOWER CONFIDENCE: 43.08
95% UPPER CONFIDENCE: 55.77

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN BNF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	35	29	40	0	0	0
MORTALITIES:	0	15	35	29	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 16.94
VAR OF LN OF EST. : .41659D-02
95% LOWER CONFIDENCE: 14.89
95% UPPER CONFIDENCE: 19.27

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(4)	20.00	27.00	60.00	86.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	35	40	0	0	0
MORTALITIES:	0	8	23	35	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 46.13
VAR OF LN OF EST. : .32569D-02
95% LOWER CONFIDENCE: 41.15
95% UPPER CONFIDENCE: 51.70

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: COUMARIN MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	36	28	12	40	0	0	0
MORTALITIES:	0	3	16	12	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 27.41
VAR OF LN OF EST. : .41478D-02
95% LOWER CONFIDENCE: 24.10
95% UPPER CONFIDENCE: 31.18

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX MIX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	36	28	32	18	0	0	0
MORTALITIES:	0	13	28	24	18	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 17.14
VAR OF LN OF EST. : .45564D-02
95% LOWER CONFIDENCE: 14.97
95% UPPER CONFIDENCE: 19.62

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	32	18	0	0	0
MORTALITIES:	0	4	26	32	18	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 25.89
VAR OF LN OF EST. : .33158D-02
95% LOWER CONFIDENCE: 23.07
95% UPPER CONFIDENCE: 29.05

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	27.00	60.00	86.00	100.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	37	7	18	0	0	0
MORTALITIES:	8	22	29	7	18	0	0	0

SPEARMAN-KARBER TRIM: 20.00%

SPEARMAN-KARBER ESTIMATES: EC50: 51.49
VAR OF LN OF EST. : .80018D-02
95% LOWER CONFIDENCE: 43.06
95% UPPER CONFIDENCE: 61.58

FETAX SUMMARY SHEET

Test No. 2	
Test Material Coumarin	Investigator Font
Source US Army SBIR	Lab SA
CAS No. Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/L

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL: No. Dead or Malformed / Total Number X 100 = %	MORTALITY RECORD	MALFORMATION RECORD
	2 : 80 X 100 = 2.5 %	____ : ____ X 100 = ____ %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		94.77	
LC_{50} 136.78/64.02/106.60/39.80/73.53		EC_{50} 42.29/19.71/48.35/20.89/34.84/53.17	
95% Confidence limits 130.63–143.23/59.41–68.99/101.89–111.52	35.11–45.11/68.42–79.09	95% Confidence Limits 37.99–47.08/16.65–23.33/43.71–53.49	18.50–23.40/31.02–39.13
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})			
91.75–97.89		47.35–59.72	

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX MORTALITY DATA

Investigator Font	**Test Material** Coumarin
Date 1/28/97	**Test Number** 2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
EX										
EX							0	0	0	
5							0	2	2	
20							0	0	0	
27							0	0	0	
UNACT 60							0	0	0	
86							0	0	0	
100							0	0	0	
115							0	0	0	
150							5	2	7	
3-NP 200							15	14	29	
5							20	20	40	
20							0	0	0	
27							0	0	0	
60							1	0	1	
↓							3	4	7	
5							20	20	40	
20							0	0	0	
1 NH 27							0	0	0	
60							0	0	0	
86							0	0	0	
100							2	3	5	
115							8	3	11	
↓							15	12	27	
							20	20	40	

FETAX MORTALITY DATA

Investigator	**Test Material** Coumarin
Date	**Test Number** 2

	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
	5							0	0	0	
mix	20							1	0	1	
	27							8	5	13	
	60							12	15	27	
	↓							20	20	40	
	5							0	0	0	
Aroclor	20							0	0	0	
	27							0	0	0	
	60							2	3	5	
	86							14	11	25	
	100							20	20	40	
	116							20	20	40	
	5							0	0	0	
	20							0	0	0	
PB	27							0	0	0	
	60							0	0	0	
	86							1	3	4	
	100							15	12	27	
	115							20	20	40	

FETAX MALFORMATION DATA

Investigator	Font	Test Material	Coumarin
Date		Test Number	2

MALFORMATION	CONCENTRATION																									TOTAL
	UNACT										BNF					INH									
	F	F	5	20	27	60	86	100	115	150	5	20	27	60	86	27	60	86	100	115	5	20				
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	1	0	0	0	8	29	40	40	33	11	2	13	30	33	—	3	25	34	29	13	0	0				
Total No.	40	38	40	40	40	40	40	40	33	11	40	40	39	33	—	40	40	35	29	13	40	40				

Comments:

FETAX MALFORMATION DATA

Investigator	Fort	Test Material	Coumarin
Date		Test Number	2

MALFORMATION	CONCENTRATION																TOTAL
	MIX				Aroclor PB												
	5	20	27	60	5	20	27	60	86	5	20	27	60	86	100		
Severe																	
Stunted																	
Gut																	
Edema																	
Multiple																	
Cardiac																	
Abdominal																	
Facial																	
Cephalic																	
Optic																	
Tail																	
Notochord																	
Fin																	
Face																	
Eye																	
Brain																	
Hemorrhage																	
Cardiac																	
Blisters																	
Other-specify																	
No. Malformed	0	7	25	13	0	1	15	20	15	0	0	6	19	28	13		
Total No.	40	39	27	13	40	40	40	35	15	40	41	41	40	36	13		

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	36	13	0	0	0
MORTALITIES:	0	6	19	28	13	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 53.17
VAR OF LN OF EST. : .33669D-02
95% LOWER CONFIDENCE: 47.35
95% UPPER CONFIDENCE: 59.72

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	.00	.00	.00	.
NUMBER EXPOSED:	40	39	27	13	0	0	0	0
MORTALITIES:	0	7	25	13	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 20.80
VAR OF LN OF EST. : .34513D-02
95% LOWER CONFIDENCE: 18.50
95% UPPER CONFIDENCE: 23.40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	35	15	0	0	0
MORTALITIES:	0	1	15	30	15	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 34.84
VAR OF LN OF EST. : .33751D-02
95% LOWER CONFIDENCE: 31.02
95% UPPER CONFIDENCE: 39.13

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN UNACT.
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	0	8	29	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 42.29
VAR OF LN OF EST. : .28794D-02
95% LOWER CONFIDENCE: 37.99
95% UPPER CONFIDENCE: 47.08

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN BNF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	39	33	40	0	0	0
MORTALITIES:	2	13	30	33	40	0	0	0	0

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: EC50: 19.71
VAR OF LN OF EST. : .71002D-02
95% LOWER CONFIDENCE: 16.65
95% UPPER CONFIDENCE: 23.33

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	35	29	0	0	0
MORTALITIES:	0	3	25	34	29	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 48.35
VAR OF LN OF EST. : .25411D-02
95% LOWER CONFIDENCE: 43.71
95% UPPER CONFIDENCE: 53.48

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	100.00	115.00	150.00	200.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	18	0	0	0
MORTALITIES:	0	7	29	40	18	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 136.78
VAR OF LN OF EST. : .52994D-03
95% LOWER CONFIDENCE: 130.63
95% UPPER CONFIDENCE: 143.23

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	18	0	0	0
MORTALITIES:	0	1	7	40	18	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 64.02
VAR OF LN OF EST. : .13949D-02
95% LOWER CONFIDENCE: 59.41
95% UPPER CONFIDENCE: 68.99

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: COUMARIN

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	150.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	5	11	27	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 106.60
VAR OF LN OF EST. : .50901D-03
95% LOWER CONFIDENCE: 101.89
95% UPPER CONFIDENCE: 111.52

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	13	27	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 39.80
VAR OF LN OF EST. : .39283D-02
95% LOWER CONFIDENCE: 35.11
95% UPPER CONFIDENCE: 45.11

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: COUMARIN

RAW DATA:

CONCENTRATION(MG/L)	27.00	60.00	86.00	100.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	5	25	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 73.53
VAR OF LN OF EST. : .12997D-02
95% LOWER CONFIDENCE: 68.42
95% UPPER CONFIDENCE: 79.03

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	4	27	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 94.77
VAR OF LN OF EST. : .26255D-03
95% LOWER CONFIDENCE: 91.75
95% UPPER CONFIDENCE: 97.89

FETAX SUMMARY SHEET

Test No.	1

Test Material 2-AAF	Investigator Font
Source US Army SBRL	Lab Stover
CAS No. Lot No.	Test Start Date 1/21/97
Composition/Purity	Test End Date 1/25/97
Solvent Conc.	Test Units (i.e., mg/ml) mg/L

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL: No. Dead or Malformed / Total Number X 100 = %	MORTALITY RECORD	MALFORMATION RECORD
	0 : 80 X 100 = 0 %	0 : 80 X 100 = 0 %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		17.70 /	
LC_{50} 125.90/75.38/17.41/100.21/17.91/		EC_{50} 8.66/12.06/7.16/10.74/6.98/7.48	
95% Confidence limits 120.22-131.81/72.33-78.59/16.64-18.25/93.73-107.13/17.41-18.42/		95% Confidence Limits 7.30-10.28/10.47-13.89/6.41-8.01/9.39-12.28/6.11-7.96/6.48-8.64	
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})			

15.90 - 19.69/

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	2-AAF
Date	1/28/97	**Test Number**	1

	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
	FETAX							0	0	0	
	FETAX							0	0	0	
	MAS - PB							0	0	0	
	CP+ PB							20	20	40	
	CP- PB							0	0	0	
	3							0	0	0	
	9.6							0	0	0	
PB	11							0	0	0	
	30							0	0	0	
	35							0	0	0	
	50							0	0	0	
	57							0	0	0	
	110							2	3	5	
	125							20	20	40	
	150							20	20	40	
	3							20	20	40	
	9.6							0	0	0	
B-NF	11							0	0	0	
	30							1	2	3	
	↓							20	20	40	
	3							20	20	40	
INH	9.6							0	0	0	
	11							0	0	0	
	30							0	0	0	
	35							0	0	0	
	50							0	0	0	
	57							0	3	3	
	110							6	7	13	
	125							20	20	40	

FETAX MORTALITY DATA

Investigator	Test Material 2-AAF
Date	Test Number 1

	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
	3										
Aroclor	9.6							0	0	0	
	11							0	0	0	
	30							1	0	1	
	↓							20	20	40	
MIX	3							20	20	40	
	9.6							0	0	0	
	11							1	1	2	
	30							15	2	17	
	35							17	16	34	
	50							19	18	37	
	57							20	20	40	
	↓							20	20	40	
								20	20	40	
UNACT	3							0	0	0	
	9.6							)	)	)	
	11										
	30										
	35										
	50										
	57										
	110							0	0	0	
	125							3	2	5	
	150							5	4	9	
								20	20	40	

FETAX MALFORMATION DATA

Investigator	Test Material 2-AAF
Date	Test Number 2

MALFORMATION	Aroclor				M/X																				TOTAL
	CONCENTRATION																								
	3	9.6	11	3	9.6	11	30	35		3	9.6	11	30	35	50	57	110	125	150						
Severe																									
Stunted																									
Gut																									
Edema																									
Multiple																									
Cardiac																									
Abdominal																									
Facial																									
Cephalic																									
Optic																									
Tail																									
Notochord																									
Fin																									
Face																									
Eye																									
Brain																									
Hemorrhage																									
Cardiac																									
Blisters																									
Other-specify																									
No. Malformed	3	23	39	2	21	28	6	3		1	11	19	39	40	40	40	35	31	—						
Total No.	40	46	39	40	38	33	6	3		40	—	—	—	—	—	40	35	31	—						

Comments:

FETAX MALFORMATION DATA

Investigator: Font	Test Material: 2-AAF
Date:	Test Number: 1

MALFORMATION	CONCENTRATION																									TOTAL
	F	F	M	CR	3	9.6	11	30	35	50	57	3	9.6	11	3	9.6	11	30	35	50	57	110				
	PB											BNF						INH								
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	5	3	5	23	40	40	40	35	1	22	37	1	10	25	40	40	40	37	27				
Total No.	40	40	40	40	40	40	40	40	40	40	35	40	40	37	40	—	—	40	40	40	37	27				

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: 2-AAF

RAW DATA:

CONCENTRATION(MG/L)	57.00	110.00	125.00	150.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	5	9	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 125.90
VAR OF LN OF EST. : .52637D-03
95% LOWER CONFIDENCE: 120.26
95% UPPER CONFIDENCE: 131.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	57.00	110.00	150.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	5	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 75.38
VAR OF LN OF EST. : .42497D-03
95% LOWER CONFIDENCE: 72.33
95% UPPER CONFIDENCE: 78.55

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	9.60	11.00	30.00	150.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	3	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 17.41
VAR OF LN OF EST. : .56294D-03
95% LOWER CONFIDENCE: 16.60
95% UPPER CONFIDENCE: 18.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	57.00	110.00	125.00	110.00	125.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	3	13	40	39	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 100.21
VAR OF LN OF EST. : .11150D-02
95% LOWER CONFIDENCE: 93.73
95% UPPER CONFIDENCE: 107.13

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: 2-AAF AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	9.60	11.00	30.00	125.00	110.00	125.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	0	1	40	40	39	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 17.91
VAR OF LN OF EST. : .19779D-03
95% LOWER CONFIDENCE: 17.41
95% UPPER CONFIDENCE: 18.42

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	2	7	34	37	40	C	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 17.70
VAR OF LN OF EST. : .28534D-02
95% LOWER CONFIDENCE: 15.90
95% UPPER CONFIDENCE: 19.69

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF UNACT.
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	1	11	19	39	40	40	0	0	0

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 8.66
VAR OF LN OF EST. : .73618D-02
95% LOWER CONFIDENCE: 7.30
95% UPPER CONFIDENCE: 10.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	3	5	23	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 12.06
VAR OF LN OF EST. : .50053D-02
95% LOWER CONFIDENCE: 10.47
95% UPPER CONFIDENCE: 13.89

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	37	40	40	40	0	0
MORTALITIES:	1	22	37	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 7.16
VAR OF LN OF EST. : .31200D-02
95% LOWER CONFIDENCE: 6.41
95% UPPER CONFIDENCE: 8.01

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: 2-AAF INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	1	10	25	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 10.74
VAR OF LN OF EST. : .45112D-02
95% LOWER CONFIDENCE: 9.39
95% UPPER CONFIDENCE: 12.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	3	23	40	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 6.98
VAR OF LN OF EST. : .43573D-02
95% LOWER CONFIDENCE: 6.11
95% UPPER CONFIDENCE: 7.96

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	.00	.00	.00	.
NUMBER EXPOSED:	40	38	33	6	0	0	0	0
MORTALITIES:	2	21	28	6	0	0	0	0

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: EC50: 7.48
VAR OF LN OF EST. : .51617D-02
95% LOWER CONFIDENCE: 6.48
95% UPPER CONFIDENCE: 8.64

FETAX SUMMARY SHEET

Test No.	2

Test Material 2-AAF	Investigator Font
Source US Army SBR	Lab SA
CAS No. Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/L

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
$\frac{\text{No. Dead or Malformed}}{\text{Total Number}}$ X 100 = %		
Solvent Control	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%
Control Length mm	Solvent Control Length mm	____ : ____ X 100 = ____%
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC_{50}		EC_{50}	
95% Confidence limits		95% Confidence Limits	
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%
2500 mg/L	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	2-AAF
Date		**Test Number**	2

	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
	FETAX							2	0	2	
	FETAX							0	1	1	
	MAS-PB							1	0	1	
	CP+							20	20	40	
	CP-							0	0	0	
	3							0	0	0	
	9.6							0	0	0	
	11							0	0	0	
PB	30							0	0	0	
	35							0	0	0	
	50							0	1	1	
	57							5	2	7	
	110							16	17	33	
	125							20	20	40	
	150							20	20	40	
	3							0	0	0	
	9.6							0	0	0	
	11							1	0	1	
BNF	30							17	12	29	
	35							20	20	40	
	50									40	
	57									40	
	110									40	
	125									40	
	150							20	20	40	
	MAS-BNF							0	1	1	
	CP+							20	20	40	
	CP-							2	1	3	

FETAX MORTALITY DATA

Investigator	**Test Material** 2-AAF
Date	**Test Number** 2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
MAS - INH							1	1	2	
AH+							18	16	34	
AH−							0	0	0	
3							0	0	0	
9.6										
11										
30										
35										
50										
57										
110										
125							1	1	2	
150							3	4	7	
3							20	20	40	
9.6							0	0	0	
11							1	0	1	
30							4	2	6	
↓							20	20	40	
3							20	20	40	
9.6							0	0	0	
11							0	1	1	
30							6	3	9	
35							15	16	31	
MAS							20	20	40	
CP+							1	0	1	
CP−							20	20	40	
							0	0	0	

INH

Arochl

FETAX MORTALITY DATA

Investigator	Test Material: 2-AAF
Date	Test Number: 2

	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
	3							0	0	0	
	9.6							3	2	5	
	11							4	4	8	
MIX	30							15	13	28	
	35							18	20	38	
	50							20	20	40	
	3							0	0	0	
	9.6										
	11										
UNACT	30										
	35										
	50										
	57							0	0	0	
	110							3	1	4	
	125							12	10	22	
	150							20	20	40	

FETAX MALFORMATION DATA

Investigator	Test Material 2-AAF
Date	Test Number 2

MALFORMATION	CONCENTRATION																								TOTAL
					PB											BNF						INH			
	F	F	M	Ctl	3	9.6	11	30	35	50	57	110		M	Ctl	3	9.6	11	80		M	AH	AH	3	9.6	
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	2	1	1	2	0	1	6	23	40	39	33	7		1	21	3	25	39	11		1	40	6	0	11	
Total No.	38	39	39	40	40	40	40	40	40	39	33	7		39	37	40	40	39	11		38	40	6	40	40	

5 3 11

Comments:

FETAX MALFORMATION DATA

Investigator	
Date	
Test Material	2-AAF
Test Number	2

INH (cont.)

MALFORMATION	CONCENTRATION																									TOTAL
	11	30	35	50	57	110	125		M	CP	3	9.6	11	30		M	CP	AA	3	9.6	11	30	35			
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	36	40	40	40	40	38	33		1	15	5	26	31	9		0	10	40	0	20	32	12	2			
Total No.	40	40	40	40	40	38	33		39	40	40	39	31	9		40	38	40	40	35	32	12	2			

Comments:

1 1

3

FETAX MALFORMATION DATA

Investigator	
Date	
Test Material	2-AAF
Test Number	2

UNACT.

MALFORMATION	CONCENTRATION																									TOTAL
	0	3	9.6	11	30	35	50	57	110	125																
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed		1	5	20	32	40	40	36	18	–																
Total No.		40	→	→	→	→	40	36	18	–																

Comments:

5

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: PB-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	1	6	23	40	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 28.08
VAR OF LN OF EST. : .33379D-02
95% LOWER CONFIDENCE: 25.02
95% UPPER CONFIDENCE: 31.52

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: BNF-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	39	40	40	40	0	0
MORTALITIES:	3	25	39	23	40	40	0	0	0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 6.72 BNF
VAR OF LN OF EST. : .42059D-02
95% LOWER CONFIDENCE: 5.90
95% UPPER CONFIDENCE: 7.65

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: INH-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	11	36	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 9.10
VAR OF LN OF EST. : .28339D-02
95% LOWER CONFIDENCE: 8.18
95% UPPER CONFIDENCE: 10.12

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: ARO-MAS
DURATION: 4 D

CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	39	31	40	40	40	0	0
MORTALITIES:	5	26	31	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 12.50%

SPEARMAN-KARBER ESTIMATES: EC50: 6.38 ARO
VAR OF LN OF EST. : .57942D-02
95% LOWER CONFIDENCE: 5.48
95% UPPER CONFIDENCE: 7.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: PB/INH/BNF-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	35	32	40	40	40	0	0
MORTALITIES:	0	20	32	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 7.09
VAR OF LN OF EST. : .29530D-02
95% LOWER CONFIDENCE: 6.36
95% UPPER CONFIDENCE: 7.90

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: UNACT
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	1	5	20	32	40	40	0	0	0

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 14.17 UNACT
VAR OF LN OF EST. : .52331D-02
95% LOWER CONFIDENCE: 12.26
95% UPPER CONFIDENCE: 16.38

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: PB-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	35.00	50.00	57.00	110.00	125.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	7	33	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 78.68
VAR OF LN OF EST. : .11536D-02
95% LOWER CONFIDENCE: 73.51
95% UPPER CONFIDENCE: 84.21

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: BNF-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	9.60	11.00	30.00	35.00	125.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	1	29	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 21.00
VAR OF LN OF EST. : .18672D-02
95% LOWER CONFIDENCE: 19.26
95% UPPER CONFIDENCE: 22.89

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: INH-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	57.00	110.00	125.00	150.00	125.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	2	7	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: INH.MAS 130.67
VAR OF LN OF EST. : .26987D-03
95% LOWER CONFIDENCE: 126.45
95% UPPER CONFIDENCE: 135.04

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: ARO-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	125.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	6	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 21.96
VAR OF LN OF EST. : .12918D-02
95% LOWER CONFIDENCE: 20.43
95% UPPER CONFIDENCE: 23.59

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: PB/INH/BNF-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	5	8	28	38	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 18.01 MIX-MS
VAR OF LN OF EST. : .42881D-02
95% LOWER CONFIDENCE: 15.80
95% UPPER CONFIDENCE: 20.53

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: UNACT
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	50.00	57.00	110.00	125.00	35.00	50.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	0	4	22	40	38	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 90.83
VAR OF LN OF EST. : .13035D-02
95% LOWER CONFIDENCE: 84.51
95% UPPER CONFIDENCE: 97.64

FETAX SUMMARY SHEET

Test No. 1	
Test Material TCE	Investigator Flint
Source Sigma / US Army	Lab SA
CAS No. Lot No. 1	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/L

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed / Total Number X 100 = %	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		412.01	
LC_{50} 278.13/190.35/390.43/412.10/181.29		EC_{50} 40.17/11.90/39.70/18.02/7.47/40.86	
95% Confidence limits 339.28–421.43/167.30–216.58/341.67–437.75 370.44–458.25/158.87–206.57		95% Confidence Limits 14.56–22.32/6.45–8.65/35.39–42.27 34.64–46.60/8.49–16.62/34.04–46.31	
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})			

370.44–458.25

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX MORTALITY DATA

Investigator Fort	**Test Material** TCE
Date	**Test Number** 1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
FETAX										
FETAX							0	0	0	
Aroclor-MAS							2	1	3	
Arroclor MAS + CP							1	0	1	
CP-							20	20	40	
5							0	0	0	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							0	0	6	
152							0	0	0	
490							0	0	0	
600							12	9	21	
INH-MAS							20	20	40	
INH + AH							0	0	0	
AH-							20	20	40	
5							5	4	9	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							0	0	0	
152							3	2	5	
490							10	6	16	
600							20	20	40	
							20	20	40	

FETAX MORTALITY DATA

Investigator Font	**Test Material** TCE
Date	**Test Number** 1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
Mixed MAS										
CP+							0	0	0	
CP−							20	20	40	
Alt+							0	0	0	
Alt−							40	20	40	
5							5	2	7	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							1	0	1	
152							3	2	5	
490							10	8	18	
600							20	20	40	
							20	20	40	
5										
16							0	0	0	
50							1	↓	↓	
85							↓			
97										
152							0	0	0	
490							6	7	13	
600							20	20	40	

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	TLE
Date		**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
B-NF - MAS										
CP+							0	0	0	
CP-							20	20	40	
5							0	0	0	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							0	0	0	
152							0	0	0	
490							1	0	1	
600							8	10	18	
PB - MAS							20	20	40	
CP+							0	0	0	
CP-							20	20	40	
5							0	1	1	
16							0	0	0	
50							0	0	0	
84							0	0	0	
97							0	0	0	
152							0	0	0	
490							0	0	0	
600							10	6	16	
							20	20	40	

FETAX MALFORMATION DATA

Investigator	Fort	Test Material	TCE
Date		Test Number	1

MALFORMATION	PB CONCENTRATION									MIX CONCENTRATION															TOTAL
	5	16	50	85	97	152	490	M	CP-	AH-	5	16	50	85	97	152		5	16	50	85	97	152	490	
Severe																									
Stunted																									
Gut																									
Edema																									
Multiple																									
Cardiac																									
Abdominal																									
Facial																									
Cephalic																									
Optic																									
Tail																									
Notochord																									
Fin																									
Face																									
Eye																									
Brain																									
Hemorrhage																									
Cardiac																									
Blisters																									
Other-specify																									
No. Malformed	3	15	40	40	40	40	27	0	0	33	10	39	40	39	35	22		0	1	21	40	40	40	27	
Total No.	40	40	40	40	40	40	24	40	40	33	40	40	40	39	35	27		40	40	40	40	40	40	27	

Comments:

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	TCE
Date		**Test Number**	1

MALFORMATION	CONCENTRATION																									
	F	F	M	CP+	5	16	50	85	97	152	490	M	AH-	5	16	50	85	97	152	490	M	CP-	5	16	50	TOTAL
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	5	0	1	21	40	40	40	19	0	31	10	23	40	40	35	24	—	0	0	0	2	21	40 39 32
Total No.	40	37	39	40	40	40	40	40	40	40	19	40	31	40	40	40	40	35	24	—	40	40	40	40	40	40 39 32

Anchor

ENJ

BNF

152 490 85

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	.00	.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	0	0	0	0	0	
MORTALITIES:	0	21	40	0	0	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 378.13
VAR OF LN OF EST. : .29384D-02
95% LOWER CONFIDENCE: 339.28
95% UPPER CONFIDENCE: 421.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/98
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	85.00	97.00	152.00	490.00	.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	0	0	0	0	
MORTALITIES:	0	5	16	40	0	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 190.35
VAR OF LN OF EST. : .41661D-02
95% LOWER CONFIDENCE: 167.30
95% UPPER CONFIDENCE: 216.58

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE B-NF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	18	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 390.13
VAR OF LN OF EST. : .33159D-02
95% LOWER CONFIDENCE: 347.69
95% UPPER CONFIDENCE: 437.75

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: TCE PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	16	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 412.01
VAR OF LN OF EST. : .28279D-02
95% LOWER CONFIDENCE: 370.44
95% UPPER CONFIDENCE: 458.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	85.00	97.00	152.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	5	18	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 181.29
VAR OF LN OF EST. : .43559D-02
95% LOWER CONFIDENCE: 158.87
95% UPPER CONFIDENCE: 206.87

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	15.00	50.00	85.00	490.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	1	21	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 40.17
VAR OF LN OF EST. : .54973D-02
95% LOWER CONFIDENCE: 34.64
95% UPPER CONFIDENCE: 46.60

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	10	23	40	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: 25.00%

SPEARMAN-KARBER ESTIMATES: LC50: 11.90
VAR OF LN OF EST. : .28590D-01
95% LOWER CONFIDENCE: 8.49
95% UPPER CONFIDENCE: 16.69

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE B-NF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	2	21	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 39.70
VAR OF LN OF EST. : .59211D-02
95% LOWER CONFIDENCE: 34.04
95% UPPER CONFIDENCE: 46.31

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	3	15	40	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 18.02
VAR OF LN OF EST. : .11409D-01
95% LOWER CONFIDENCE: 14.56
95% UPPER CONFIDENCE: 22.32

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 39 10
CHEMICAL: TCE MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	10	39	40	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: 25.00%

SPEARMAN-KARBER ESTIMATES: EC50: 7.47
VAR OF LN OF EST. : .53655D-02
95% LOWER CONFIDENCE: 6.45
95% UPPER CONFIDENCE: 8.65

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	1	21	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 40.86
VAR OF LN OF EST. : .51548D-02
95% LOWER CONFIDENCE: 35.39
95% UPPER CONFIDENCE: 47.17

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	85.00	490.00	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	16	40	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 412.01
VAR OF LN OF EST. : .28279D-02
95% LOWER CONFIDENCE: 370.44
95% UPPER CONFIDENCE: 458.25

FETAX SUMMARY SHEET

Test No. 2	
Test Material TCE	Investigator Font
Source Sigma / US Army SBIR	Lab S+A
CAS No. Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/L

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed / Total Number X 100 = %	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC_{50} 383.49 / 175.27 / 388.93 / 410.75 / 169.11 / 426.40		EC_{50} 31.29 / 11.45 / 40.22 / 19.46 / 7.60 / 36.23	
95% Confidence limits 341.64 – 430.47 / 153.43 – 200.22 / 344.62 – 438.94 / 367.06 – 459.63 / 147.80 – 198.49		95% Confidence Limits 32.04 – 43.40 / 8.65 – 15.16 / 34.17 – 47.34 / 10.41 – 13.07 / 6.35 – 9.19 / 30.84 – 42.56	
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})			

384.46 – 472.92

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX MALFORMATION DATA

Investigator: Fort	Test Material: TCE
Date:	Test Number: 2

MALFORMATION	Aroclor CONCENTRATION												ENT CONCENTRATION													TOTAL 152 490
	F	F	M	CP	5	16	50	85	97	152	490	M	Alt	5	16	50	85	97	152	M	CP	5	16	50	85	
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	2	2	1	3	0	2	24	40	40	40	39	21	40	8	25	40	40	32	21	0	2	0	3	19	40	38 23
Total No.	39	38	40	36	40	40	40	40	40	40	39	21	40	40	40	40	40	32	21	40	40	40	40	40	40	38 23

Comments:

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	TCE
Date		**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
Mixed MAS							0	0	0	
CP+							20	20	40	
CP−							3	4	7	
AH+							18	17	35	
AH−							5	2	7	
5							0	0	0	
16							0	0	0	
50							0	0	0	
85							1	0	1	
97							5	4	9	
152							12	8	20	
490							20	20	40	
600							20	20	40	
5							0	0	0	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							0	0	0	
152							0	0	0	
490							8	6	14	
600							20	20	40	

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	TCE
Date		**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
FETAX							0	1	1	
FETAX							1	1	2	
Aroclor MAS							0	0	0	
CP+							20	20	40	
CP-							3	1	4	
5							0	0	0	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							0	0	0	
152							1	0	1	
490							10	9	19	
600							20	20	40	
INH-MAS							0	0	0	
AH+							20	20	40	
AH-							3	4	7	
5							0	0	0	
16							0	0	0	
50							0	0	0	
85							0	0	0	
97							4	4	8	
152							10	9	19	
490							20	20	~~40~~ 40	
							↓	↓	~~40~~	

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	TCE
Date		**Test Number**	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%		
B-NF MAS							0	0	0			
CP+							20	20	40			
CP-							2	3	5			
5							0	0	0			
16							0	0	0			
50							0	0	0			
85							0	0	0			
97							0	0	0			
152							1	1	2			
490							8	9	17			
600							20	20	40			
PB-MAS							1	0	1			
CP+							20	20	40			
CP-							3	1	4			
5							0	0	0			
16							0	0	0			
50							0	0	0			
85							0	0	0			
97							0	0	0			
152							1	0	1			
490							7	8	15			
600							20	20	40			

FETAX MALFORMATION DATA

Investigator: Fort	Test Material: TCE
Date:	Test Number: 2

MALFORMATION	CONCENTRATION																									TOTAL
	PB									MIX									no MAS							
	M	CP-	5	16	50	85	97	152	490	M	CP-	AH-	5	16	50	85	97	152	5	16	50	85	97	152	490	
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	13	40	40	40	39	25	0	0	33	11	36	40	39	31	20	0	3	24	40	40	40	26	
Total No.	40	36	40	40	40	40	40	39	25	40	33	33	40	40	40	39	31	20	40				→	40	26	

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	2	17	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 388.93
VAR OF LN OF EST. : .36583D-02
95% LOWER CONFIDENCE: 344.62
95% UPPER CONFIDENCE: 438.94

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	15	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 410.75
VAR OF LN OF EST. : .31613D-02
95% LOWER CONFIDENCE: 367.06
95% UPPER CONFIDENCE: 459.63

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	85.00	97.00	152.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	9	20	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 169.11
VAR OF LN OF EST. : .45342D-02
95% LOWER CONFIDENCE: 147.80
95% UPPER CONFIDENCE: 193.49

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	19	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 383.49
VAR OF LN OF EST. : .33380D-02
95% LOWER CONFIDENCE: 341.64
95% UPPER CONFIDENCE: 430.47

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/7/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: TCE

RAW DATA:

CONCENTRATION(MG/L)	85.00	97.00	152.00	490.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	8	19	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 175.27
VAR OF LN OF EST. : .44267D-02
95% LOWER CONFIDENCE: 153.43
95% UPPER CONFIDENCE: 200.22

CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	152.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	14	40	20	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 426.40
VAR OF LN OF EST. : .26806D-02
95% LOWER CONFIDENCE: 384.46
95% UPPER CONFIDENCE: 472.92

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	8	25	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 20.00%

SPEARMAN-KARBER ESTIMATES: EC50: 11.45
VAR OF LN OF EST. : .19686D-01
95% LOWER CONFIDENCE: 8.65
95% UPPER CONFIDENCE: 15.16

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE aN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	19	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 40.22
VAR OF LN OF EST. : .66460D-02
95% LOWER CONFIDENCE: 34.17
95% UPPER CONFIDENCE: 47.34

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: TCE

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	13	40	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 19.46
VAR OF LN OF EST. : .72694D-02
95% LOWER CONFIDENCE: 16.41
95% UPPER CONFIDENCE: 23.07

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE NO MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	3	24	40	0	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 36.23
VAR OF LN OF EST. : .64825D-02
95% LOWER CONFIDENCE: 30.84
95% UPPER CONFIDENCE: 42.56

SBIR Unactivated Definitive

UNACTIVATED Cyclophosphamide

Concentration (mg/mL) FX Control 5.5 6-AN 2500 6-AN	FX (mL)	Stock (mL)
3.5	4.706	3.294
4	4.235	3.765
4.5	3.765	4.235
5	3.294	4.706
5.5	2.824	5.176
6	2.353	5.647
6.5	1.882	6.118
7	1.412	6.588
7.5	0.941	7.059
8	0.471	7.529
8.5	0.000	8.000
	Sum	62.118
	x 2 dishes	124.2353
	x 4 days	496.9412

8.5 mg/mL x 500mL

= 4,250 g

UNACTIVATED Trichlorethylene

Concentration (mg/mL) FX Control 5.5 6-AN 2500 6-AN	FX (mL)	Stock (mL)
0.035	7.533	0.467
0.04	7.467	0.533
0.045	7.400	0.600
0.05	7.333	0.667
0.055	7.267	0.733
0.06	7.200	0.800
0.065	7.133	0.867
0.3	4.000	4.000
0.35	3.333	4.667
0.4	2.667	5.333
0.45	2.000	6.000
0.5	1.333	6.667
0.55	0.667	7.333
0.6	0.000	8.000
	Sum	46.667
	x 2 dishes	93.33333
	x 4 days	373.3333

0.6 mg/mL x 500 mL

= 300 mg

UNACTIVATED 2-AAF

Concentration (mg/mL) FX Control 5.5 6-AN 2500 6-AN	FX (mL)	Stock (mL)
0.02	7.360	0.640
0.025	7.200	0.800
0.03	7.040	0.960
0.035	6.880	1.120
0.04	6.720	1.280
0.045	6.560	1.440
0.05	6.400	1.600
0.09	4.960	3.040
0.1	4.800	3.200
0.15	3.200	4.800
0.2	1.600	6.400
0.25	0.000	8.000
	Sum	33.280
	x 2 dishes	66.56
	x 4 days	266.24

0.25 mg/mL x 500 mL

= 125 mg

mg/mL Coumarin

UNACTIVATED 2-AAF

Concentration (mg/mL) FX Control 5.5 6-AN 2500 6-AN	FX (mL)	Stock (mL)
0.045	6.560	1.440
0.05	6.400	1.600
0.055	6.240	1.760
0.06	6.080	1.920
0.065	5.920	2.080
0.07	5.760	2.240
0.075	5.600	2.400
0.095	4.960	3.040
0.1	4.800	3.200
0.15	3.200	4.800
0.2	1.600	6.400
0.25	0.000	8.000
	Sum	38.880
	x 2 dishes	77.76
	x 4 days	311.04

0.05 mg/L x 0.5 L

= 0.025 mg

11

Unactivated Definitive range test 2-AAF

~~Carcinogenic Mechanism of Action I~~

9-23-96

UNACTIVATED 2-AAF DEFINITIVE						
Concentration (mg/L)	FX (mL)	Stock (mL)	MAS (mL)	Generator (mL)	Enzyme (mL)	Other
FX Controls	8					
5.5 6-AN Control						
2500 6-AN Control						
0.02	6.933	1.067				
0.025	6.667	1.333				
0.03	6.400	1.600				
0.035	6.133	1.867				
0.04	5.867	2.133				
0.045	5.600	2.400				
0.05	5.333	2.667				
0.09	3.200	4.800				
0.095	2.933	5.067				
0.1	2.667	5.333				
0.125	1.333	6.667				
0.15	0.000	8.000				

~~Thalidomide Mechanism of Action I~~
~~9-25-06~~

THIOACETAMIDE UNACTIVATED RANGE							
Concentration (mg/mL)	FX (mL)	Stock A (mL)	Stock B (mL)	MAS (mL)	Generator (mL)	Enzyme (mL)	Other
FX Controls	8						
5.5 6-AN Control							
2500 6-AN Control							
0.005	7.920		0.080				
0.01	7.840		0.160				
0.05	7.200		0.800				
0.1	6.400		1.600				
0.5	0.000		8.000				
1	7.680	0.320					
2.5	7.200	0.800					
5	6.400	1.600					
10	4.800	3.200					
25	0.000	8.000					

Stock solution A=~~8~~ 25 mg/mL
Total volume of stock A required to finish one (1) test=
111.36 mL

Stock solution B=~~8~~ 0.5 mg/mL
Total volume of stock B required to finish one (1) test=
85.12

25 mg/ml x 200ml = 5000mg

FETAX SUMMARY SHEET

Test No.	1

Test Material: Cyclophosphamide	Investigator: Fort
Source: Sigma Chemical	Lab: SA
CAS No. / Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent / Conc.	Test Units (i.e., mg/ml): mg/mL

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL: No. Dead or Malformed / Total Number X 100 = %	MORTALITY RECORD	MALFORMATION RECORD
	0 : 80 X 100 = 0 %	0 : 80 X 100 = 0 %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length ____ mm	Solvent Control Length ____ mm	
Minimum Concentration to Inhibit Growth (MCIG)		

no MAs, β-NF, INH, Aroclor, PB, mix

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC_{50} > 4.0, 3.09, >4.0, 1.50, 1.19, 1.34		EC_{50} > 4.0, 1.81, >4.0, 0.59, 0.61, 0.40	
95% Confidence limits ND, 3.0-3.17, ND 1.02-1.38, 1.61-1.81, 1.26-1.43		95% Confidence Limits ND, 1.72-1.91, ND 0.51-0.68, 0.53-0.70, 0.34-0.47	
TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})		ND, 1.71, ND, 2.54, 2.79, 3.35	

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
2500 mg/L	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	Cyclophosphamide
Date	12/4/96	**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
FETAX							0	0	0	
FETAX							0	0	0	
PB - MAS							0	0	0	
B-NF - MAS							0	0	0	
INH - MAS							0	0	0	
Aroclor - MAS							0	0	0	
Mix - MAS							0	0	0	
0.1							0	0	0	
0.5										
0.75										
1										
no MAS 1.25										
1.5										
2										
2.5										
3										
3.5							1	0	1	
4							3	2	5	
0.1							0	0	0	
0.5							0	0	0	
B-NF 0.75							0	0	0	
1							0	0	0	
1.25							0	0	0	
1.5							0	0	0	
2							0	0	0	
2.5							2	1	3	
3							3	5	8	
3.5							20	20	40	
4							20	20	40	

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	CPA
Date	12/4/96	**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
0.1							0	0	0	
0.5										
0.75										
1										
1.25										
1.5										
2.0										
2.5							2	1	3	
3							5	3	8	
3.5							5	2	7	
4							9	5	14	
0.1							0	0	0	
0.5							0	0	0	
0.75							0	0	0	
1							2	1	3	
1.25							3	4	7	
1.5							12	9	21	
2.0							18	17	35	
2.5							20	20	40	
3							20	20	40	
3.5							20	20	40	
4							20	20	40	

INH

Aroclor

FETAX MORTALITY DATA

Investigator	Fort	Test Material	CPA
Date	12/4/96	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
0.1							0	0	0	
0.5							0	0	0	
0.75							2	0	2	
1							5	3	8	
MIX 1.25							8	3	11	
1.5							12	10	22	
2							20	20	40	
2.5										
3										
3.5										
4										
0.1							0	0	0	
0.5							0	0	0	
PB 0.75							0	0	0	
1							0	1	1	
1.25							4	3	7	
1.5							8	5	13	
2							10	14	24	
2.5							20	20	40	
3							20	20		
3.5										
4										

CP - Aro MAS	0	0	0
CP - INH MAS	0	0	0
CP - BNF MAS	0	0	0
CP - PB MAS	2	1	3
CP - MIX MAS	1	0	1

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	CPA
Date	12/4/96	**Test Number**	

MAS — CP- — CONCENTRATION — NO MAS

MALFORMATION	F	F	BNF	INH	PB	A	M	BNF	INH	AB	A	M	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4			TOTAL
Severe																										
Stunted																					1	6	10			
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	10			
Total No.	40	40	40	40	40	40	40	40	40	37	40	39	40	—							→	39	35			

Comments:

FETAX MALFORMATION DATA

Investigator	Ford	**Test Material**	CRA
Date		**Test Number**	1

B-NF — CONCENTRATION — INH

MALFORMATION	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4				TOTAL
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	0	1	10	23	37	32	-	-	0	0	0	0	0	0	1	5	61	6	10				
Total No.	40	40	40	40	40	40	40	37	32	-	-	40	40	40	40	40	40	40	37	33	32	26				

Comments:

FETAX MALFORMATION DATA

Investigator Font	**Test Material** CPA
Date	**Test Number** 1

MALFORMATION	CONCENTRATION Aroclor										MIX							PB								TOTAL
	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	0.1	0.5	0.75	1	1.25	1.5	2	0.1	0.5	0.75	1	1.25	1.5	2		
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	8	23	35	33	9	5	–	–	–	0	18	35	32	19	8	–	0	8	18	39	33	27	16		
Total No.	40	40	40	37	33	9	5	–	–	–	40	40	38	22	19	8	–	40	40	40	39	33	27	16		

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA B-NF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	2.00	2.50	3.00	3.50	4.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	8	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.09
VAR OF LN OF EST. : .18450D-03
95% LOWER CONFIDENCE: 3.00
95% UPPER CONFIDENCE: 3.17

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

VOID

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CP INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	2.00	2.50	3.00	3.50	4.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	8	27	38	0	0	0	0

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.28
VAR OF LN OF EST. : .33661D-03
95% LOWER CONFIDENCE: 3.16
95% UPPER CONFIDENCE: 3.40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.75	1.00	1.25	1.50	2.00	2.50	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	3	7	21	35	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.50
VAR OF LN OF EST. : .78417D-03
95% LOWER CONFIDENCE: 1.42
95% UPPER CONFIDENCE: 1.58

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: CPA - PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.75	1.00	1.25	1.50	2.00	2.50	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	1	7	13	24	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.70
VAR OF LN OF EST. : .88239D-03
95% LOWER CONFIDENCE: 1.61
95% UPPER CONFIDENCE: 1.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L (M)	.50	.75	1.00	1.25	1.50	2.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	2	8	11	22	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.34
VAR OF LN OF EST. : .95015D-03
95% LOWER CONFIDENCE: 1.26
95% UPPER CONFIDENCE: 1.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA - BNF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.00	1.25	1.50	2.00	2.50	2.00	.00	.
NUMBER EXPOSED:	40	40	40	40	37	40	0	0
MORTALITIES:	0	1	10	23	37	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 1.81
VAR OF LN OF EST. : .68247D-03
95% LOWER CONFIDENCE: 1.72
95% UPPER CONFIDENCE: 1.91

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA - INH
SPECIES: FETAX

X VOID

RAW DATA:

CONCENTRATION(MG/ML)	1.50	2.00	2.50	3.00	3.50	2.00	.00	.
NUMBER EXPOSED:	40	40	37	32	13	40	0	0
MORTALITIES:	0	1	5	19	13	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 2.83
VAR OF LN OF EST. : .38293D-03
95% LOWER CONFIDENCE: 2.73
95% UPPER CONFIDENCE: 2.95

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA - AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.10	.50	.75	1.00	1.25	2.00	.00	.
NUMBER EXPOSED:	40	40	40	37	35	40	0	0
MORTALITIES:	0	8	23	35	35	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: .59
VAR OF LN OF EST. : .48838D-02
95% LOWER CONFIDENCE: .51
95% UPPER CONFIDENCE: .68

FETAX SUMMARY SHEET

Test No. 2	
Test Material Cyclophosphamide	Investigator Font
Source Sigma Chemical	Lab S+A
CAS No. Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/mL

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed / Total Number X 100 = %		
	2 : 80 X 100 = 2.5 %	2 : 78 X 100 = ___ %
Solvent Control MAS	0 : 40 X 100 = 0 %	3 : 40 X 100 = 7.5 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

unactivated/B-NF/INH/Aroclor/PB/mix

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC_{50} >4,00/3.13/>4.00/1.85/1.59/1.69		EC_{50} >4,00/1.86/>4,00/0.71/0.92/0.72	
95% Confidence limits N.D/3.00-3.28/ND/1,74-1,98 1,51-1,67/1,58-1.81		95% Confidence Limits 1.15-1.97/ND/0,62-0.80/ 0,87-0,97/0,63-0.81	

TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50}) ND/1.68/ND/2.61/1.73 2.35

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2500 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	CP (B-NF - MAS)
Date	12/30/96	**Test Number**	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
~~FETAX~~										
FETAX										
MAS										
CP+							0	1	1	
CP−							20	20	40	
							1	1	2	
0.1										
0.5							0	1	1	
0.75							0	0	0	
1							0	0	0	
1.25							0	0	0	
1.5							0	0	0	
2							0	0	0	
2.5							0	2	2	
3							2	4	6	
3.5							7	7	14	
4							10	11	21	
							20	20	40	

FETAX MALFORMATION DATA

Investigator	Font	**Test Material**	CP (BNF - MAS)
Date	12/30/96	**Test Number**	2

MALFORMATION	CONCENTRATION																										TOTAL
	F	F	M	Ctr		0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5												
Severe			1	38																							
Stunted																											
Gut																											
Edema																											
Multiple																											
Cardiac																											
Abdominal																											
Facial																											
Cephalic																											
Optic																											
Tail																											
Notochord																											
Fin																											
Face																											
Eye																											
Brain																											
Hemorrhage																											
Cardiac																											
Blisters																											
Other-specify																											
No. Malformed			1	38		0	0	0	0	3	10	20	30	26	19												
Total No.			39	38		40	40	40	40	40	40	38	34	26	19												

Comments:

FETAX MORTALITY DATA

Investigator: Fort	Test Material: CP (INH-MAS)
Date: 12/20/96	Test Number: 2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
~~MAS~~							0	0	0	
CP+							20	20	40	
CP-							0	0	0	
0.1										
0.5							0	0	0	
0.75										
1										
1.25										
1.5										
2.0										
2.5										
3										
3.5							0	0	0	
4							3	2	5	
							7	8	15	

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	CP (INH-MAS)
Date	12/31/96	**Test Number**	2

MALFORMATION	CONCENTRATION																TOTAL
	M		CP-		0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4		
Severe																	
Stunted																	
Gut																	
Edema																	
Multiple																	
Cardiac																	
Abdominal																	
Facial																	
Cephalic																	
Optic																	
Tail																	
Notochord																	
Fin																	
Face																	
Eye																	
Brain																	
Hemorrhage																	
Cardiac																	
Blisters																	
Other-specify																	
No. Malformed	0	40			0	—	—	—	—	—	→	3	5	5	10		
Total No.	40	40			40	—	—	—	—	—	→	40	35	25			

Comments:

FETAX MORTALITY DATA

Investigator	For +	**Test Material**	CY (Aroclor MAS)
Date	12/30/96	**Test Number**	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
MAS							1 : 0	1	
CY+							20 : 20	40	
CY-							1 : 2	3	
0.1							0 : 0	0	
0.5							0 : 0	0	
0.75							0 : 0	0	
1							0 : 0	0	
1.25							1 : 4	5	
1.5							8 : 6	14	
2							12 : 11	23	
2.5							15 : 12	27	
3							20 : 20	40	
3.5							20 : 20	40	
4							20 : 20	40	

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	CP (Aroclor - MAS)
Date	12/3/96	**Test Number**	2

MALFORMATION	CONCENTRATION																										TOTAL
	M		CP-		0.1	0.5	0.75	1	1.25	1.5	2	2.5															
Severe	1		25																								
Stunted																											
Gut																											
Edema																											
Multiple																											
Cardiac																											
Abdominal																											
Facial																											
Cephalic																											
Optic																											
Tail																											
Notochord																											
Fin																											
Face																											
Eye																											
Brain																											
Hemorrhage																											
Cardiac																											
Blisters																											
Other-specify																											
No. Malformed	1		25		0	5	18	28	35	26	17	13															
Total No.	39		37		40	40	40	40	35	26	17	13															

Comments:

FETAX MORTALITY DATA

Investigator	First	Test Material	CP PB-MAS
Date	12/30/96	Test Number	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
MAS							0	0	0	
CPF							20	20	40	
UP-							0	0	0	
0.1							0	0	0	
0.5							0	0	0	
0.75							0	0	0	
1							0	1	1	
1.25							3	2	5	
1.5							8	8	16	
2							17	17	34	
2.5							20	20	40	
3							20	20	40	
3.5							20	20	40	
4							20	20	40	

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	CP (PB-MAS)
Date	12/30/96	**Test Number**	2

MALFORMATION	CONCENTRATION																									TOTAL
	M		CP-		0.1	0.5	0.75	1	1.25	1.5	2															
Severe			30																							
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0		30		0	0	5	23	35	14	6															
Total No.	40		40		40	40	40	39	35	14	6															

Comments:

FETAX MORTALITY DATA

Investigator: Fort	Test Material: Cyclophosphamide mix
Date: 12/30/96	Test Number: 2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
FETAX										
FETAX							1	1	2	
MAS							0	0	0	
CP+							0	0	0	
CP-							20	20	40	
							1	2	3	
0.1										
0.5							0	0	0	
0.75							0	0	0	
1							0	0	0	
1.25							2	0	2	
1.5							4	2	6	
2							8	12	20	
2.5							10	15	25	
3							16	16	32	
3.5							20	20	40	
4							20	20	40	
							20	20	40	

FETAX MORTALITY DATA

Investigator	Font	**Test Material**	CP (unactivated)
Date	12/30/96	**Test Number**	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%	
FETAX							1	1	2		
FETAX							0	0	0		
0.1							0	0	0		
0.5											
0.75											
1.0											
1.25											
1.5											
2											
2.5											
3											
3.5							0	1	1		
4							2	3	5		

FETAX MALFORMATION DATA

Investigator Font	**Test Material** CP (unmetabolized)
Date 12/30/96	**Test Number** 2

MALFORMATION	CONCENTRATION																										TOTAL
	F	F		0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4													
Severe	1	1																									
Stunted																											
Gut																											
Edema																											
Multiple																											
Cardiac																											
Abdominal																											
Facial																											
Cephalic																											
Optic																											
Tail																											
Notochord																											
Fin																											
Face																											
Eye																											
Brain																											
Hemorrhage																											
Cardiac																											
Blisters																											
Other-specify																											
No. Malformed	1	1		0	0	0	0	0	0	0	0	1	2	5													
Total No.	39	39		40	—	—	—	—	—	—	—	→	39	35													

Comments:

FETAX MALFORMATION DATA

Investigator	Font	**Test Material**	CP (Mix)
Date	12/31/91	**Test Number**	2

MALFORMATION	CONCENTRATION												TOTAL
	M		CP-	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	
Severe	2		37										
Stunted													
Gut													
Edema													
Multiple													
Cardiac													
Abdominal													
Facial													
Cephalic													
Optic													
Tail													
Notochord													
Fin													
Face													
Eye													
Brain													
Hemorrhage													
Cardiac													
Blisters													
Other-specify													
No. Malformed	2		37	0	5	16	27	34	20	15	8	—	
Total No.	40		37	40	40	40	38	34	20	15	8	—	

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP B-NF MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.50	2.00	2.50	3.00	3.50	4.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	2	6	14	21	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.13
VAR OF LN OF EST. : .49844D-03
95% LOWER CONFIDENCE: 3.00
95% UPPER CONFIDENCE: 3.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.00	1.25	1.50	2.00	2.50	3.00	.00	.
NUMBER EXPOSED:	40	40	40	38	34	26	0	0
MORTALITIES:	0	3	10	20	30	26	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 1.86
VAR OF LN OF EST. : .88363D-03
95% LOWER CONFIDENCE: 1.75
95% UPPER CONFIDENCE: 1.97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP AROCLOR-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.00	1.25	1.50	2.00	2.50	3.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	5	14	23	27	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.85
VAR OF LN OF EST. : .10504D-02
95% LOWER CONFIDENCE: 1.74
95% UPPER CONFIDENCE: 1.98

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP AROCLOR MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.10	.50	.75	1.00	1.25	3.00	.00	.
NUMBER EXPOSED:	40	40	40	40	35	40	0	0
MORTALITIES:	0	5	18	28	35	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: .71
VAR OF LN OF EST. : .38610D-02
95% LOWER CONFIDENCE: .62
95% UPPER CONFIDENCE: .80

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP PB-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.75	1.00	1.25	1.50	2.00	2.50	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	1	5	16	34	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.59
VAR OF LN OF EST. : .69143D-03
95% LOWER CONFIDENCE: 1.51
95% UPPER CONFIDENCE: 1.67

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D

CHEMICAL: CP PB-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.50	.75	1.00	1.25	.00	.00	.00	.
NUMBER EXPOSED:	40	40	39	35	0	0	0	0
MORTALITIES:	0	5	23	35	0	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: .92
VAR OF LN OF EST. : .73314D-03
95% LOWER CONFIDENCE: .87
95% UPPER CONFIDENCE: .97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP MIX-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.75	1.00	1.25	1.50	2.00	2.50	3.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	2	6	20	25	32	40	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.69
VAR OF LN OF EST. : .11003D-02
95% LOWER CONFIDENCE: 1.58
95% UPPER CONFIDENCE: 1.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP MIX-MAS
SPECIES: FETAX

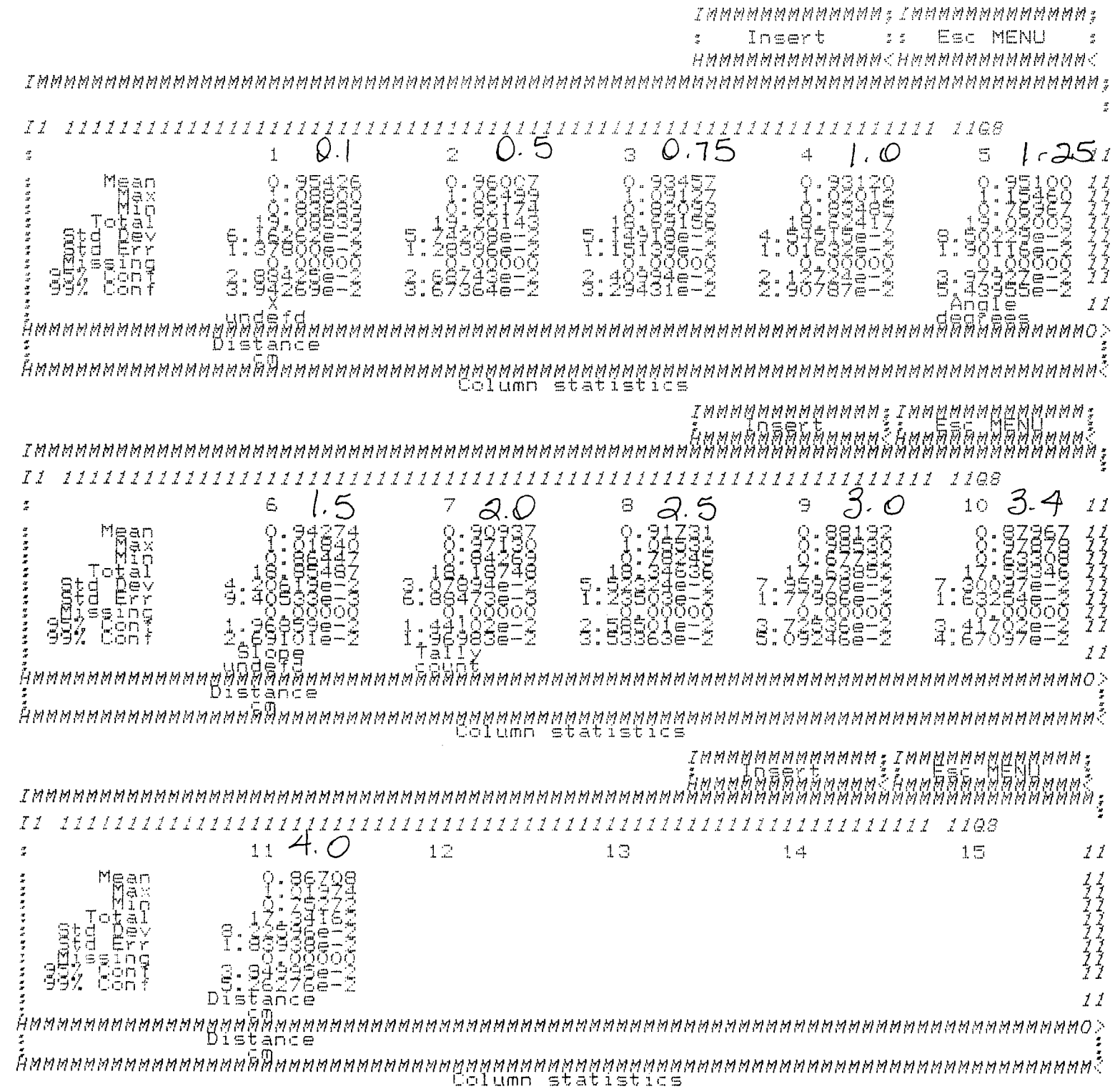
RAW DATA:

CONCENTRATION(MG/ML)	.10	.50	.75	1.00	1.25	2.50	3.00	.
NUMBER EXPOSED:	40	40	40	38	34	40	40	0
MORTALITIES:	0	5	16	27	34	32	40	0	0

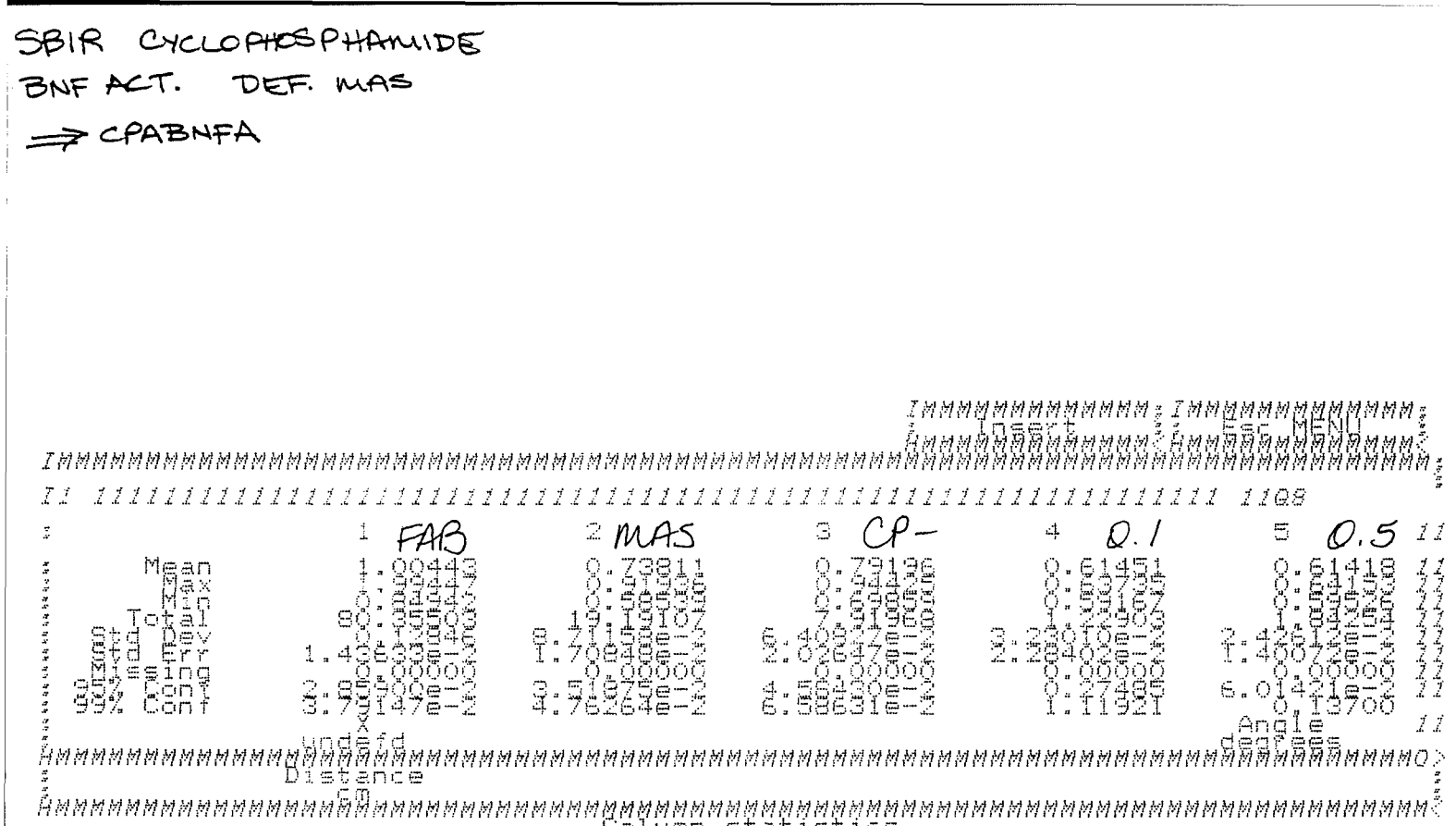
SPEARMAN-KARBER TRIM: .00%

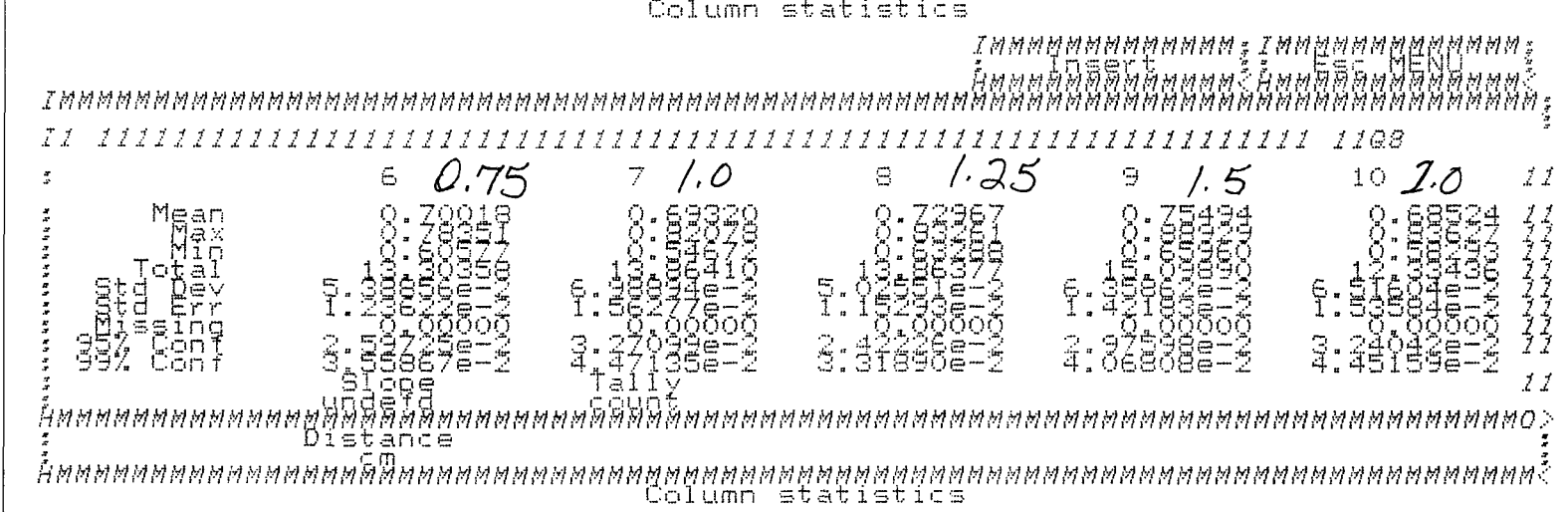
SPEARMAN-KARBER ESTIMATES: EC50: .72
VAR OF LN OF EST. : .38491D-02
95% LOWER CONFIDENCE: .63
95% UPPER CONFIDENCE: .81

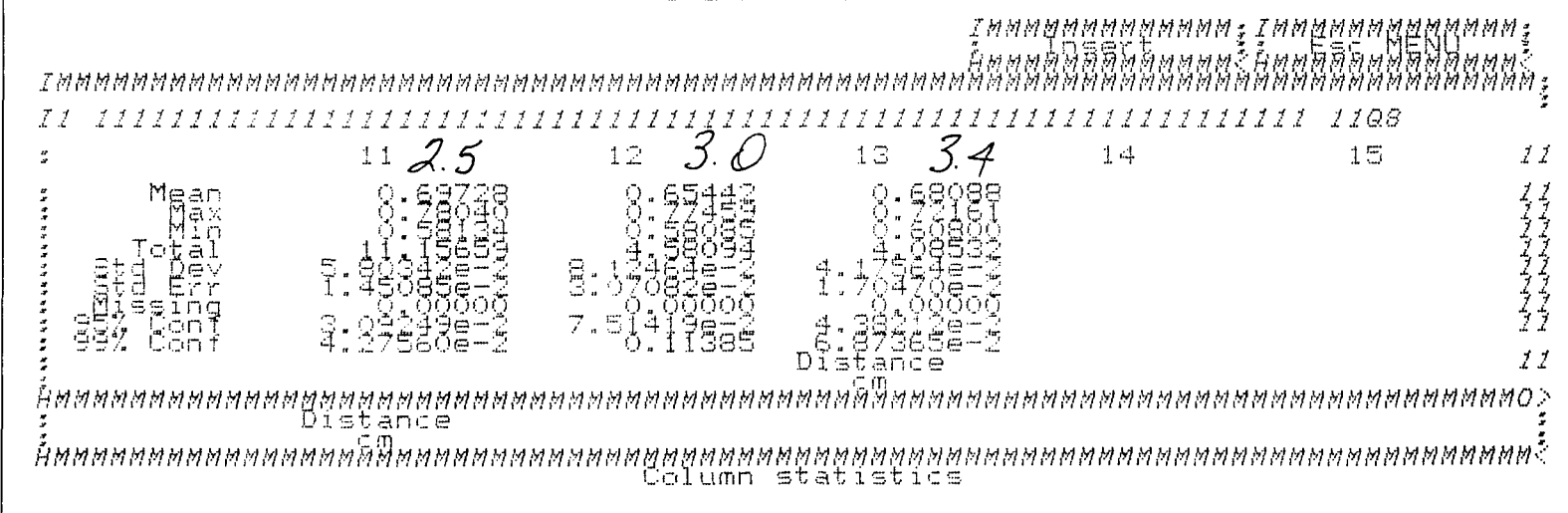
SBIR
CYCLOPHOSPHAMIDE
DEF. MAS UNACTIVATED
⇒CPADUA



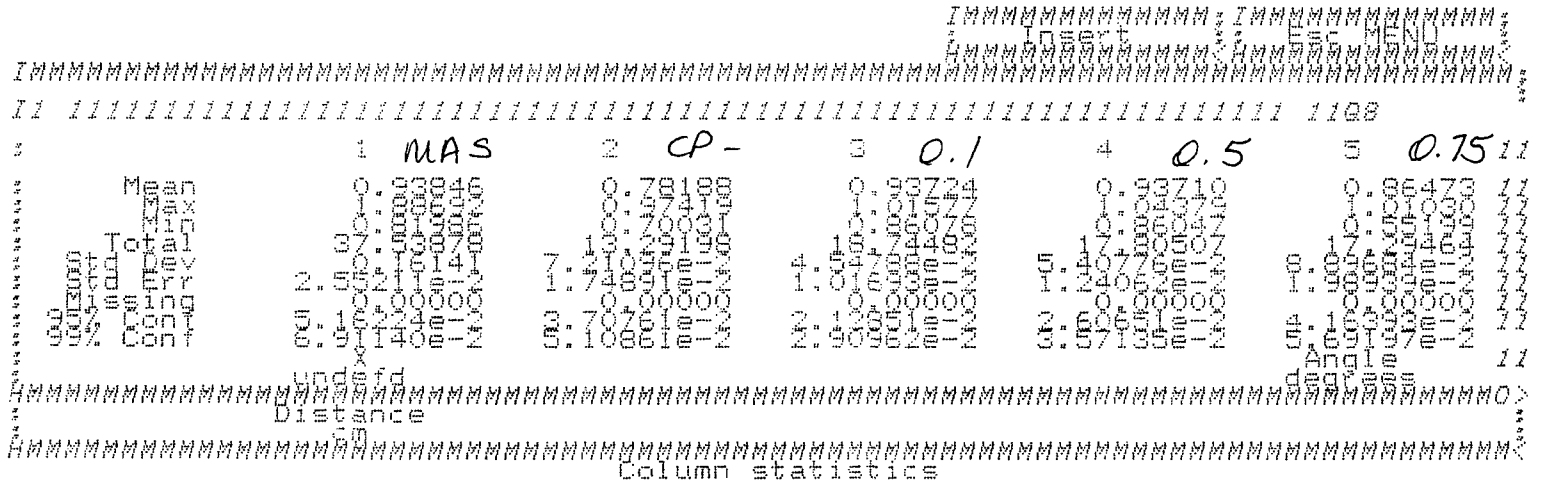
SBIR CYCLOPHOSPHAMIDE
BNF ACT. DEF. MAS
⇒ CPABNFA

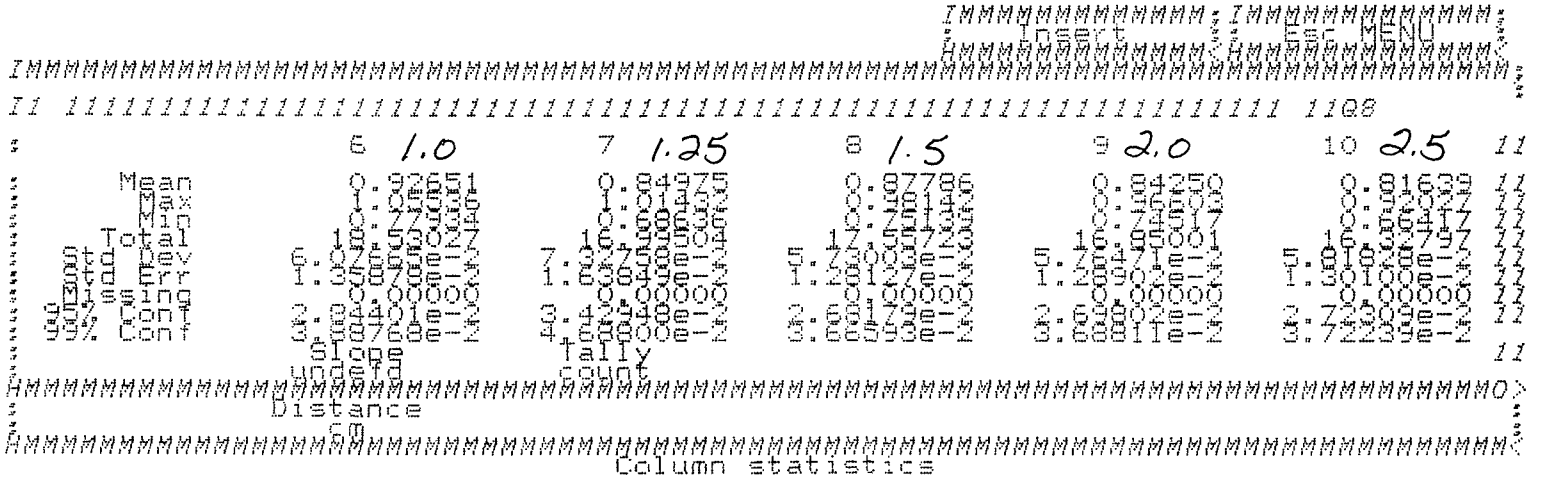


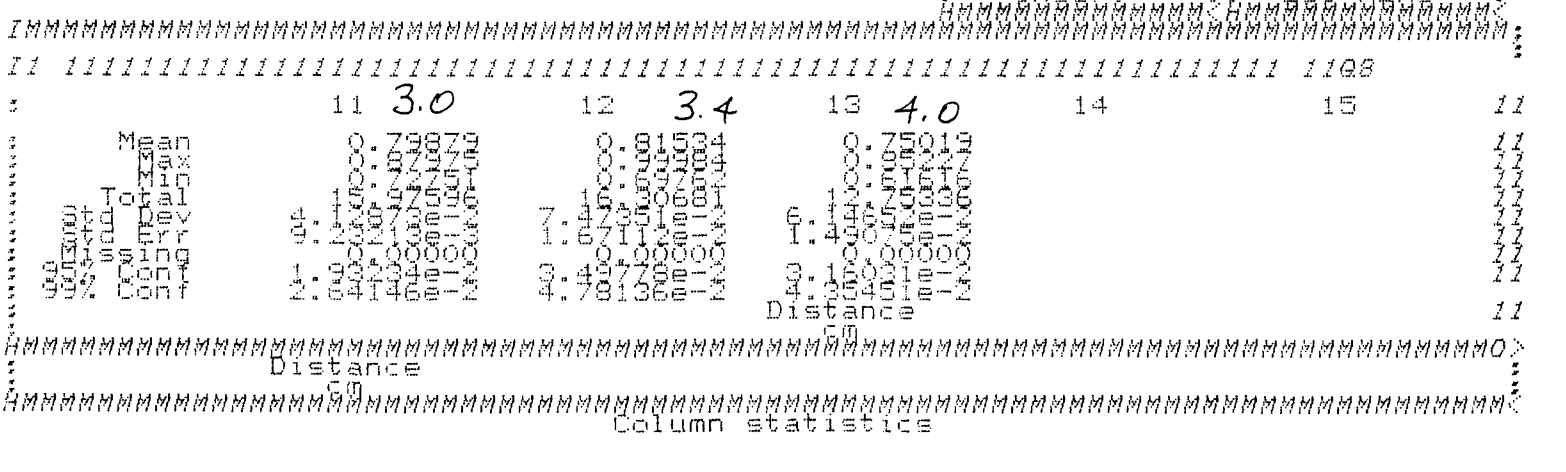




5BIR CYCLOPHOSPHAMIDE
INH ACT. DEF MAS
⇒ CPA INHA



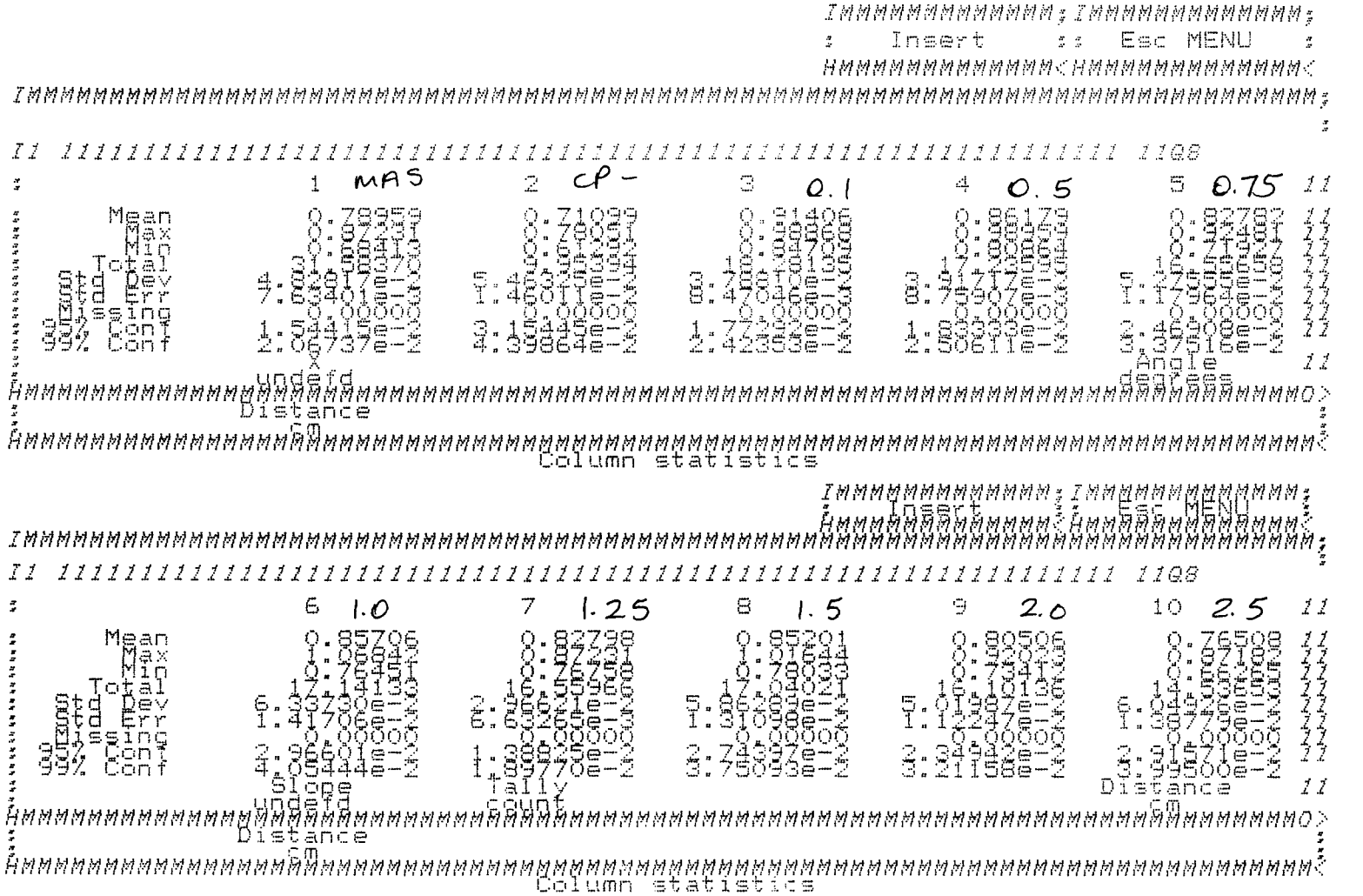




SBIR CYCLOPHOSPHAMIDE

ARO ACT. DEF MAS

⇒ CPAARDA

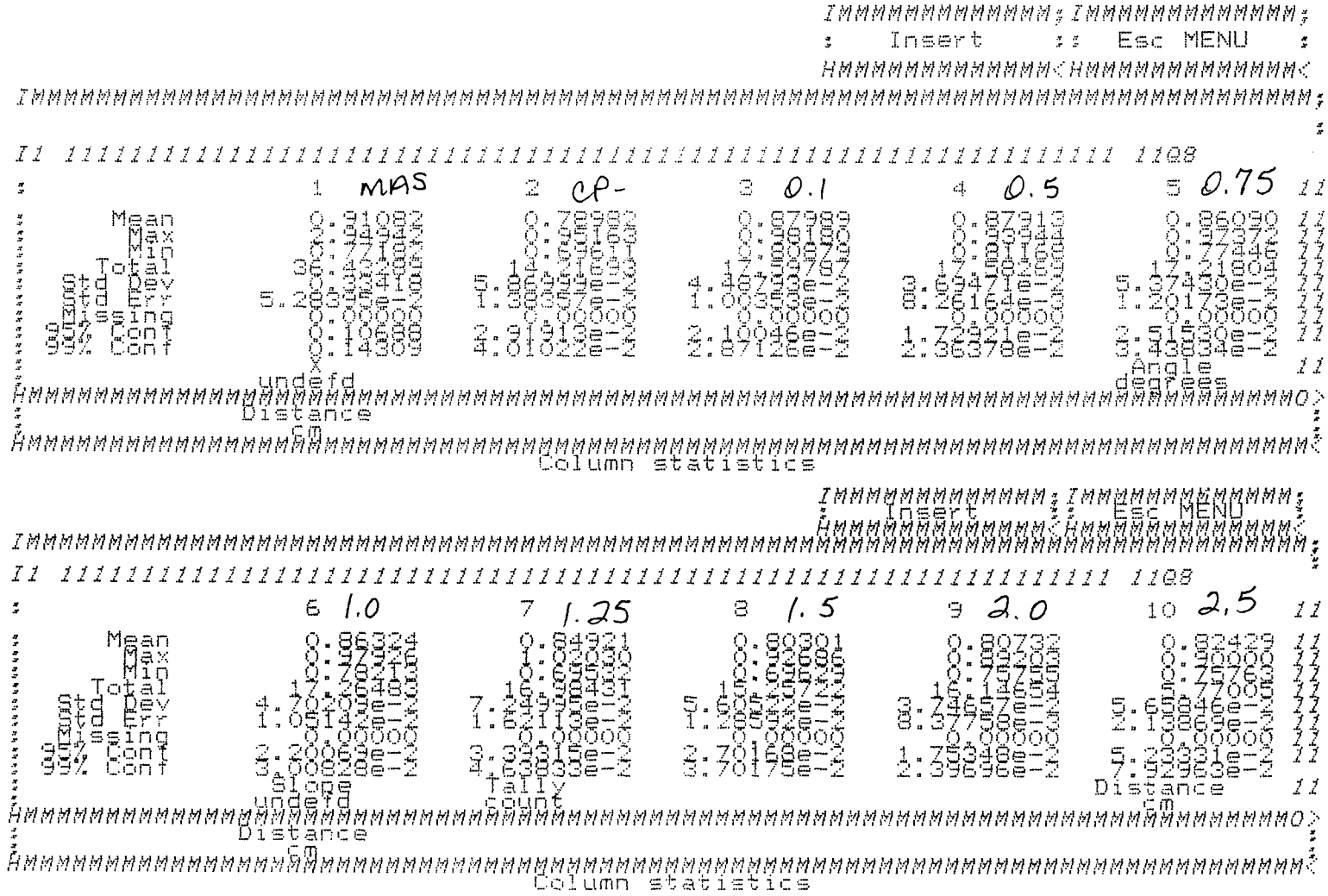


SBIR CYCLOPHOSPHAMIDE

PB ACT

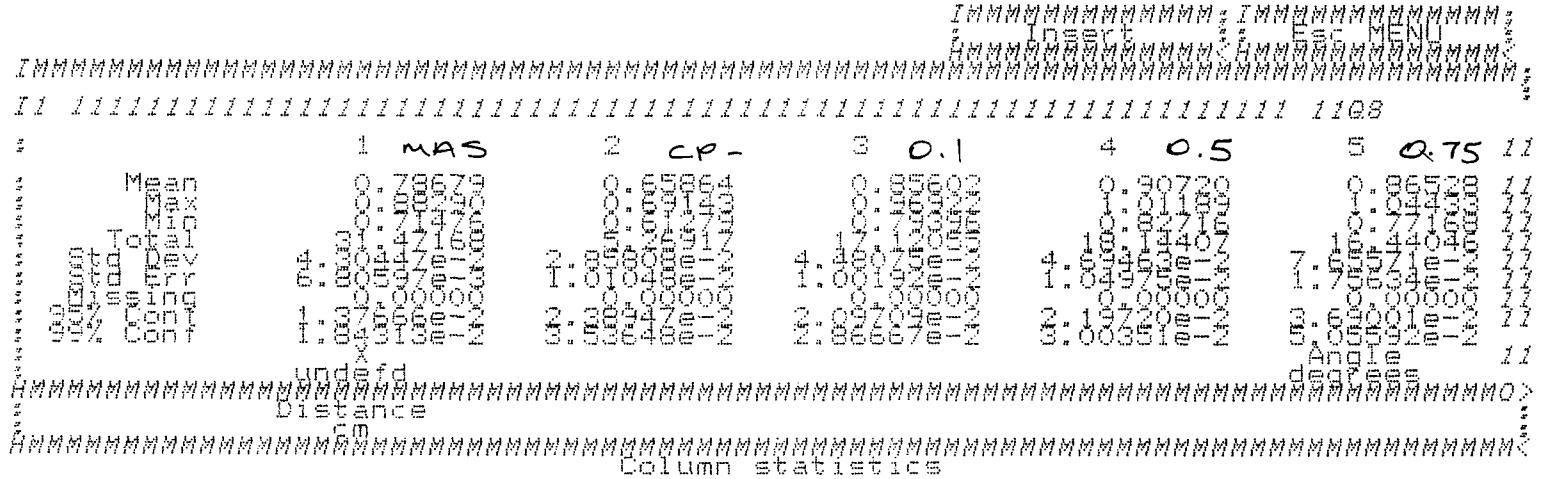
DEF. MAS

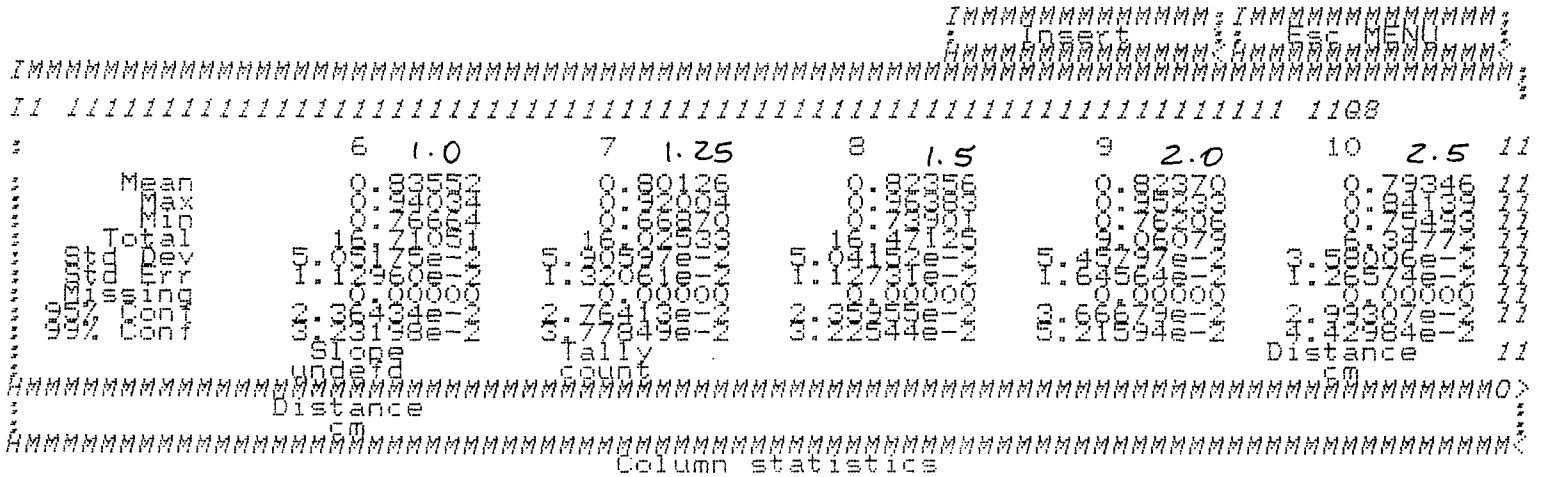
⇒ CPAPBA



SBIR CYCLOPHOSPHAMIDE
DEF MAS BNF/INH/PB ACT.

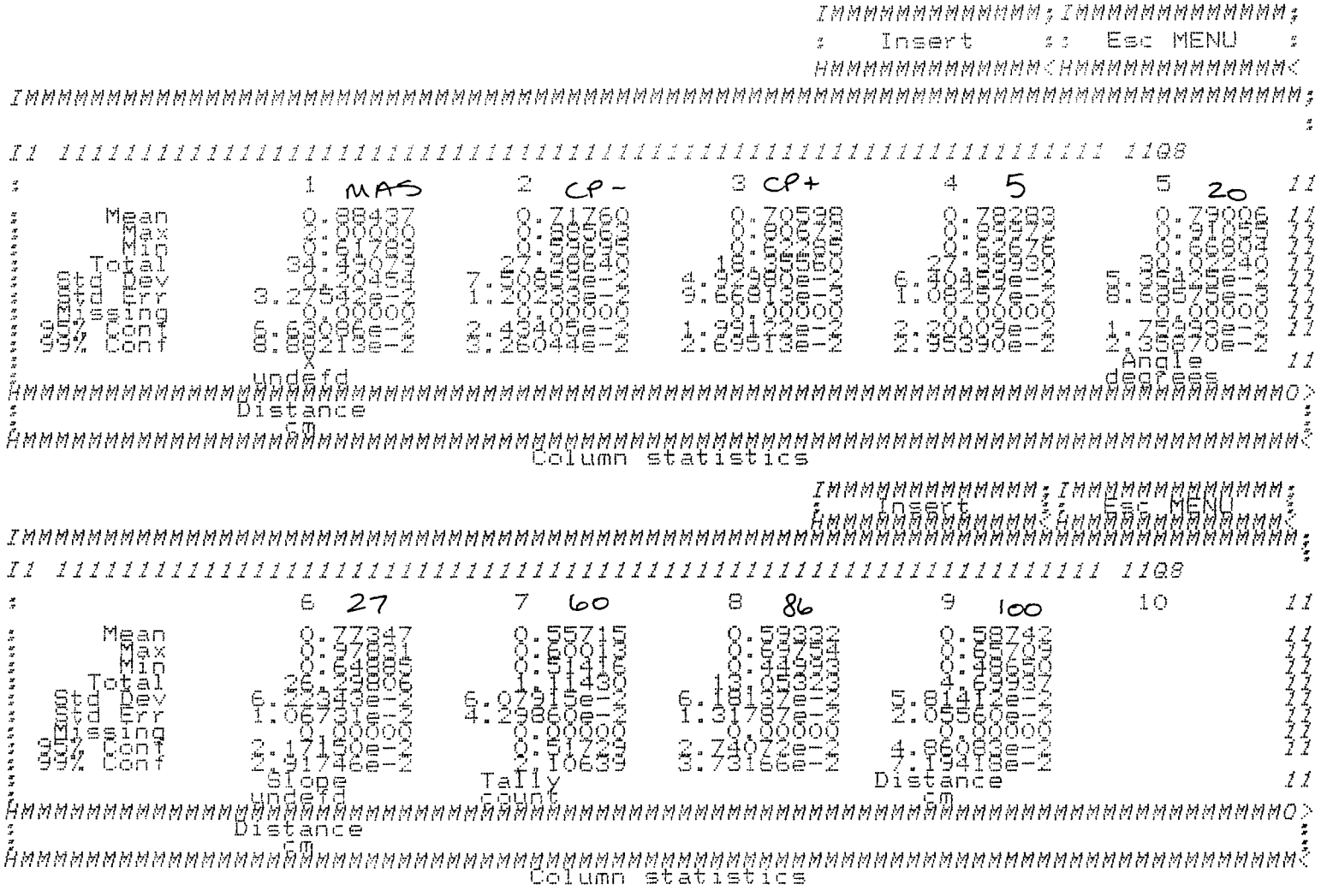
⇒ BNFINHPB





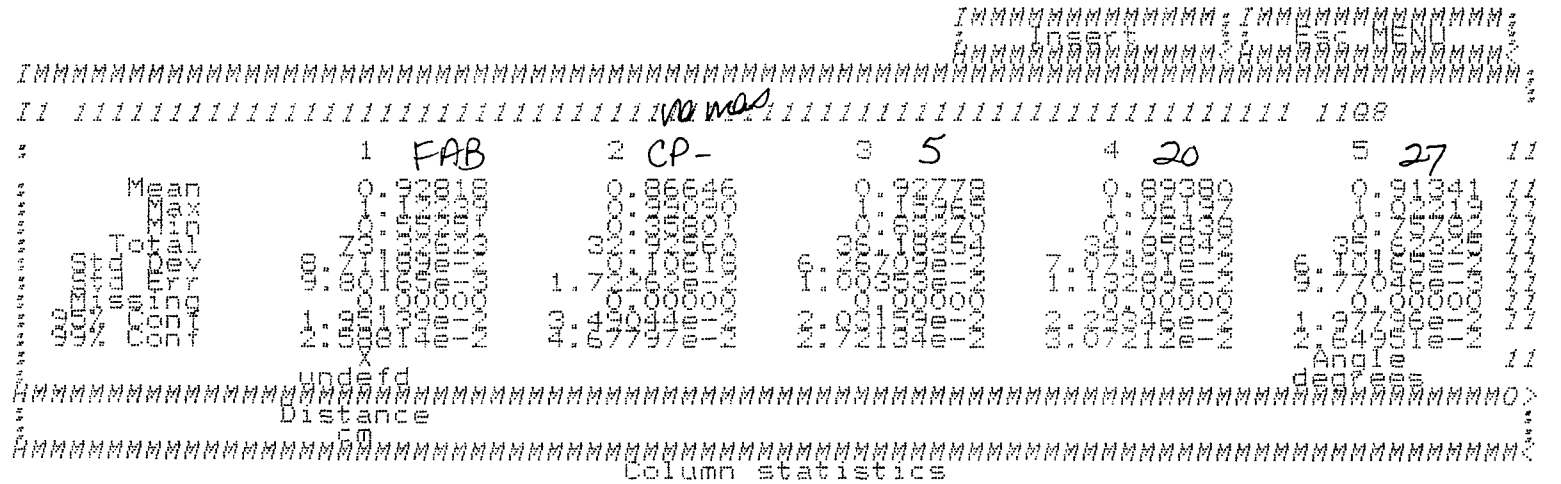
SBIR COUMARIN 2 AROCHLOR ACT. 1-13-97

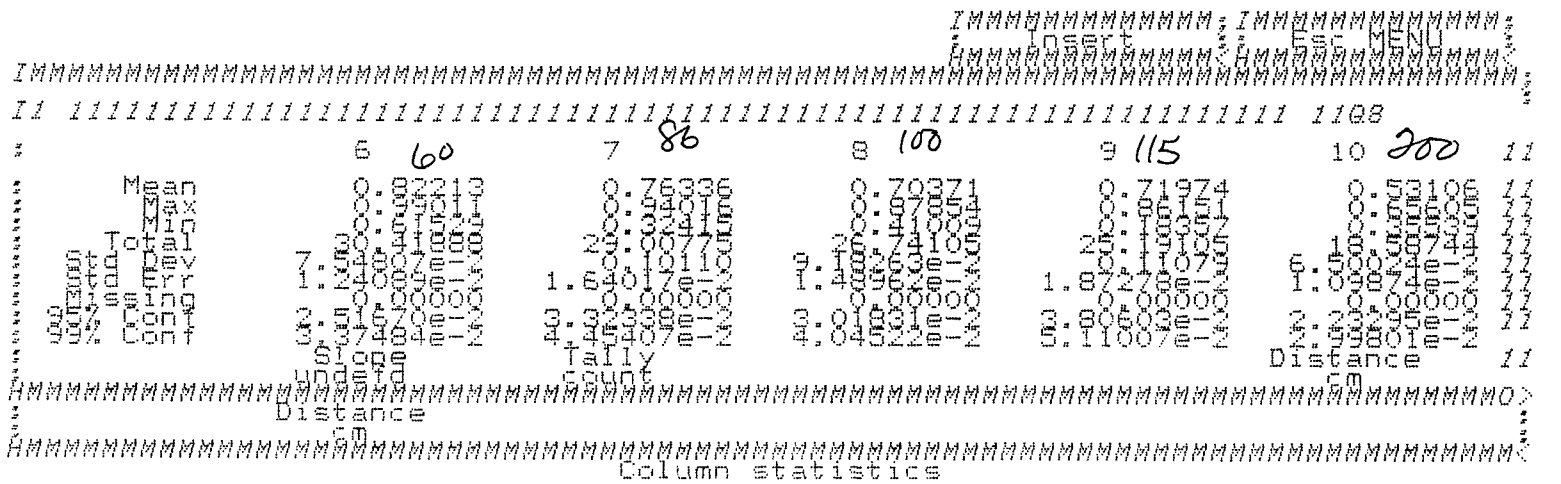
⇒ C2AROA



SBIR COUMARIN II (THE SEQUEL) UA 1-13-97

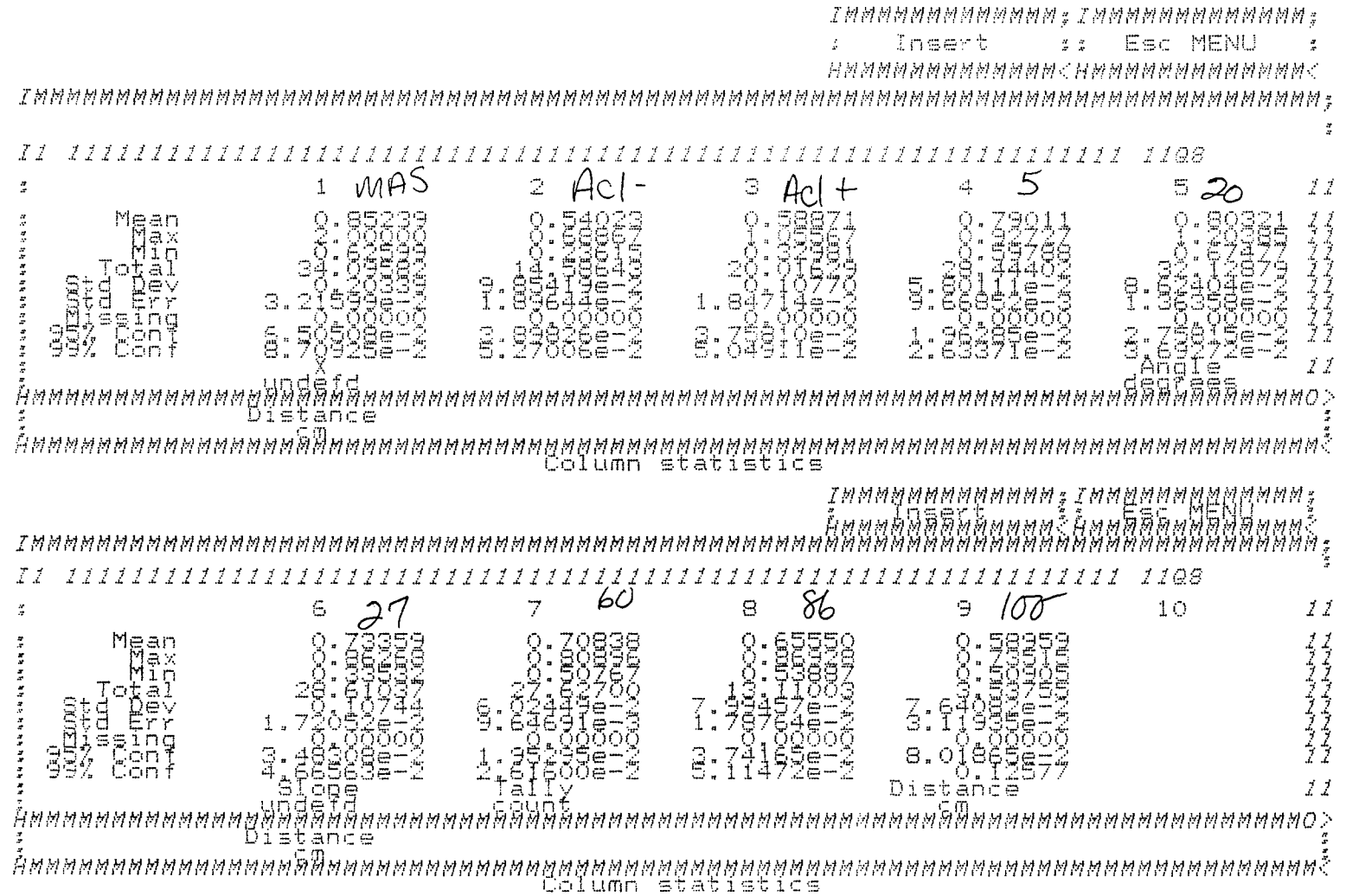
⇒C2UA





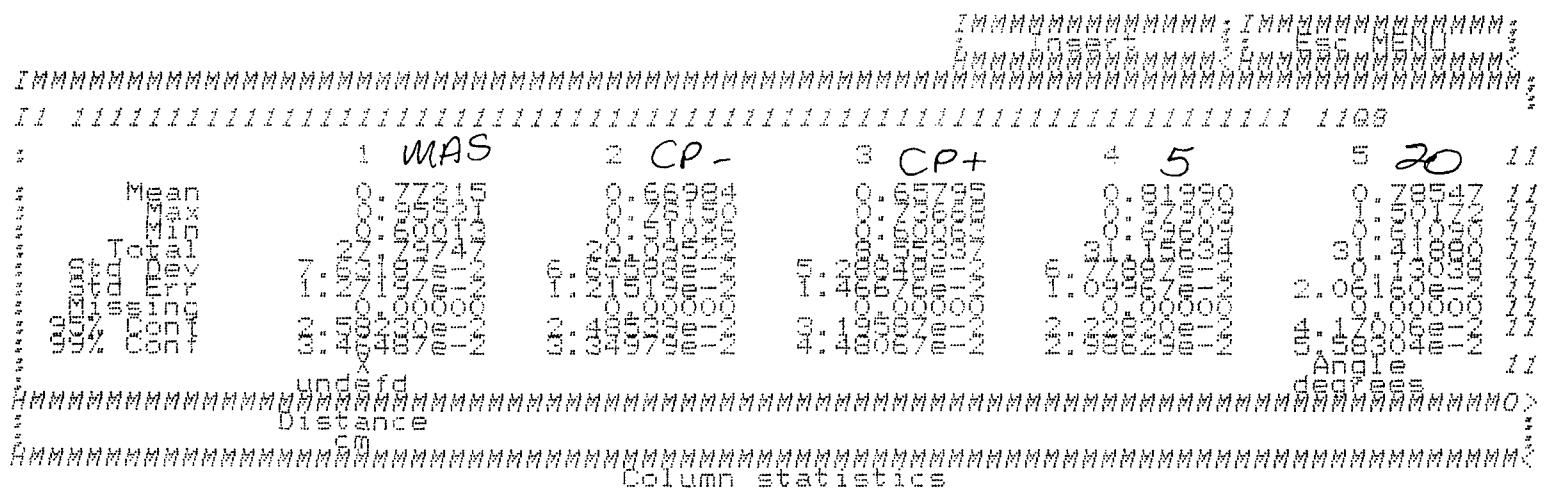
SBIR COUMARIN 2 INH ACTIVATED 1-13-97

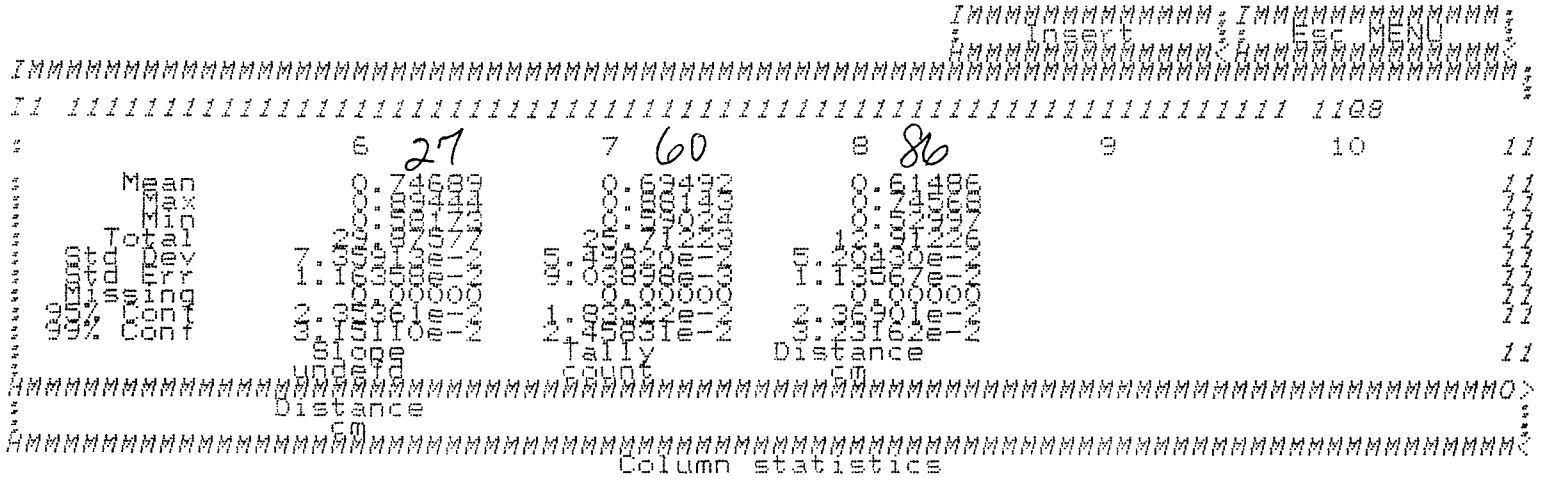
⇒C2INHA



SBIR COUMARIN 2 BNF ACT. 1·13·97

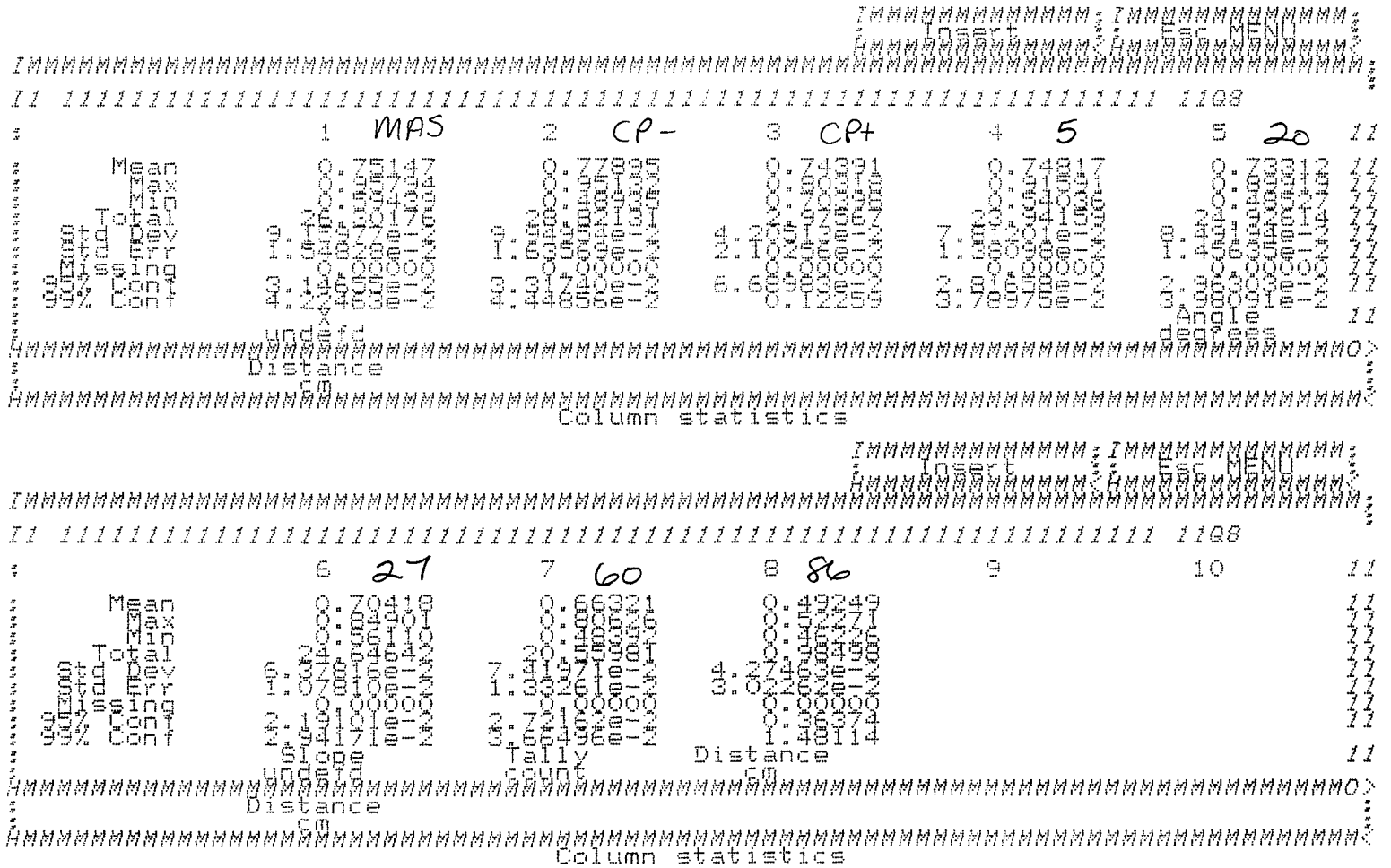
⇒C2BNFA

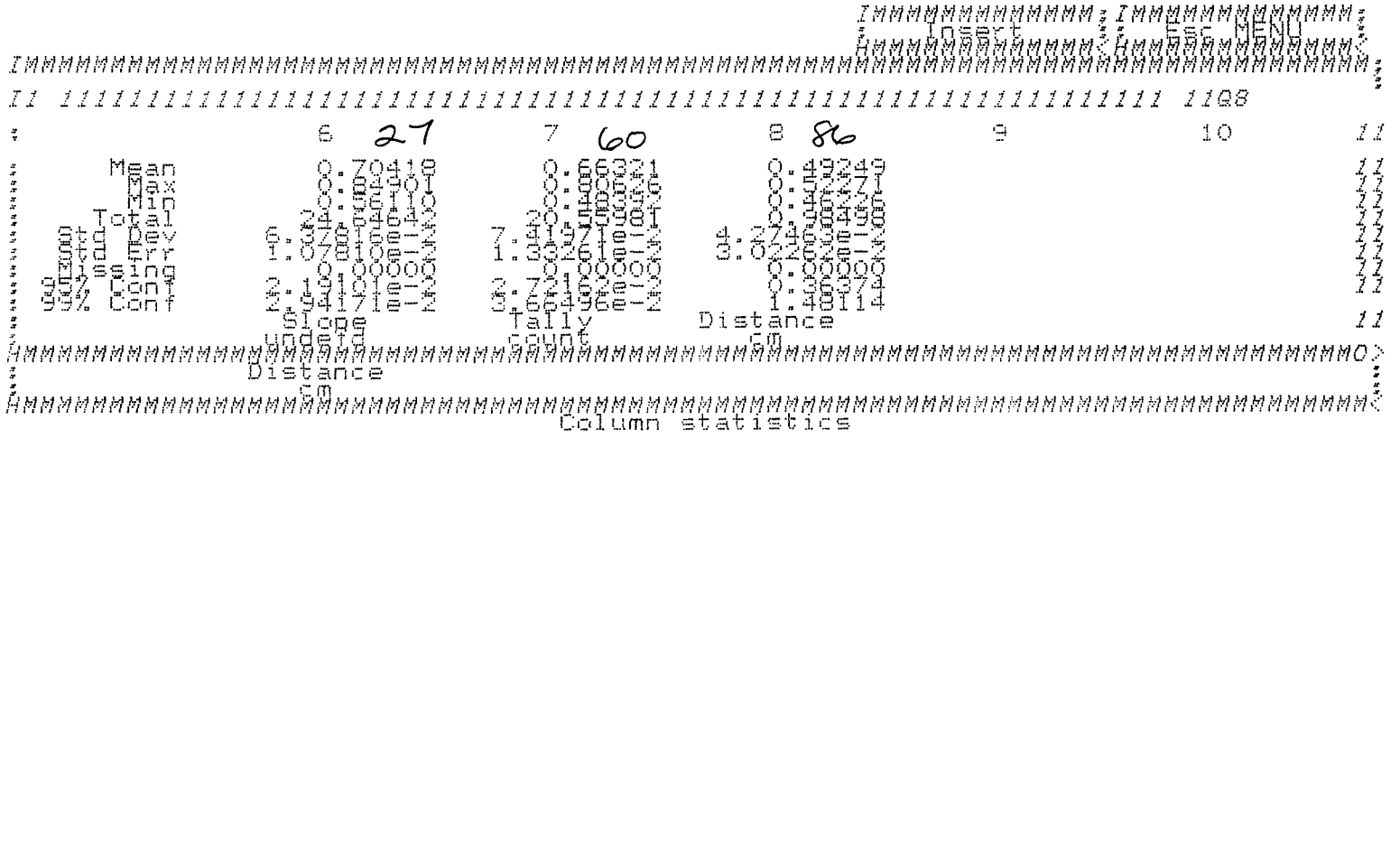




SBIR COUMARIN 2 MIX ACT. 1-13-97

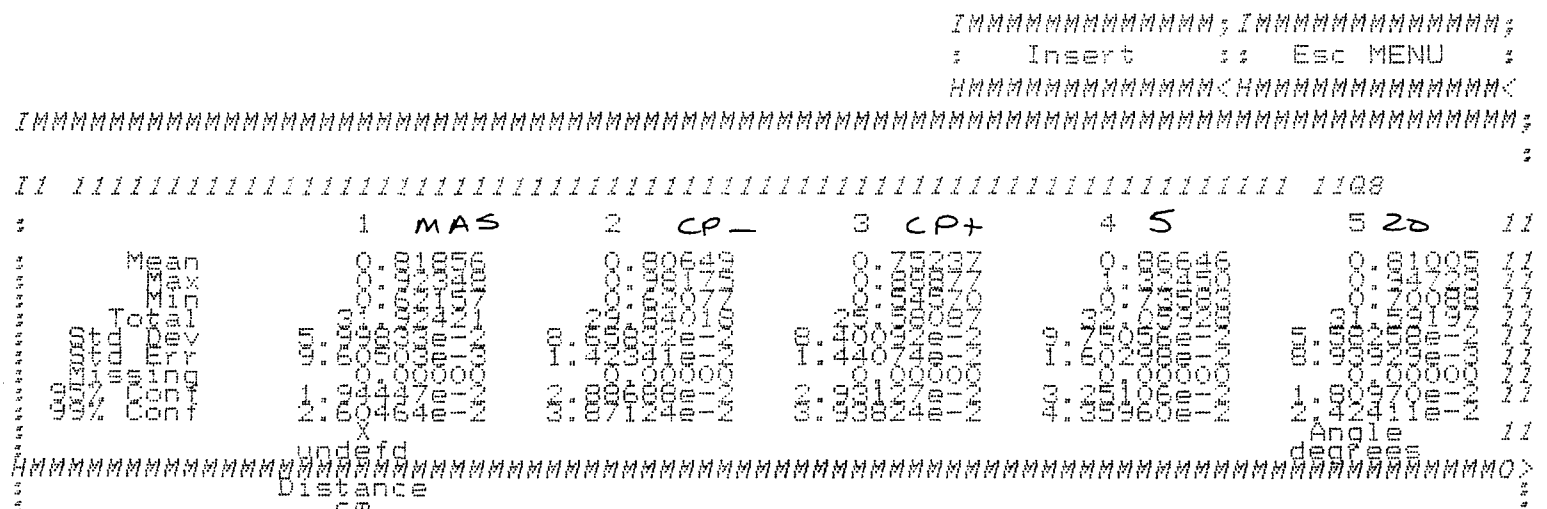
⇒ C2MIXA

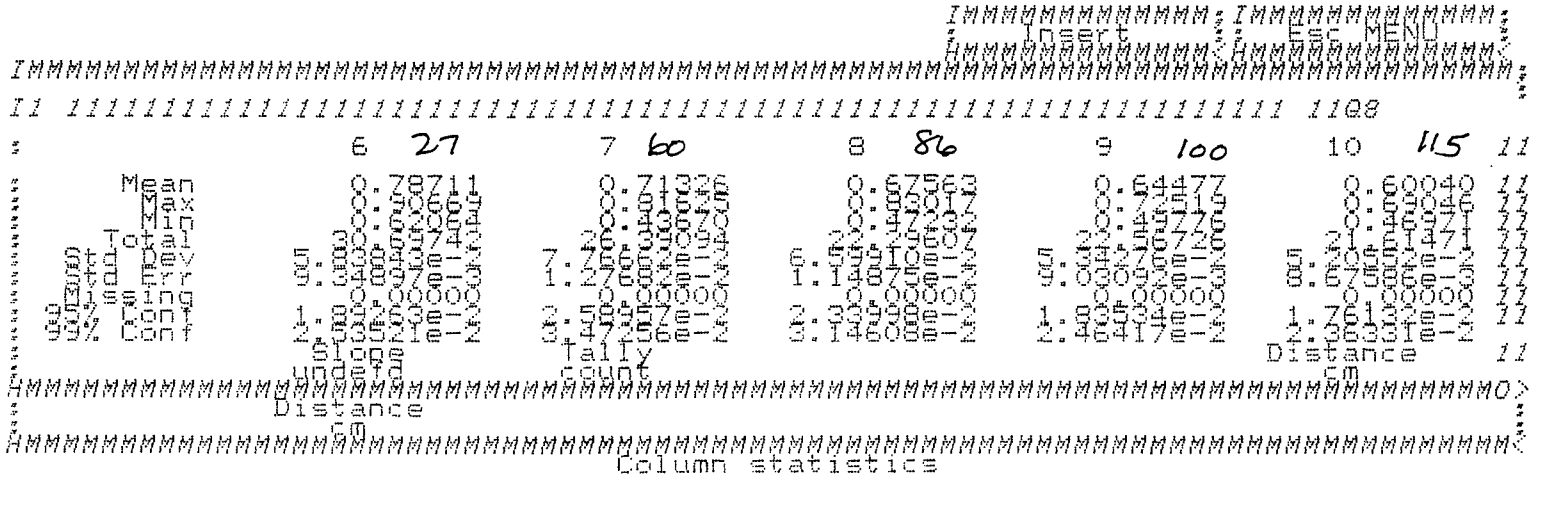




SBIR Coumarin 2 PB ACT 1-13-97

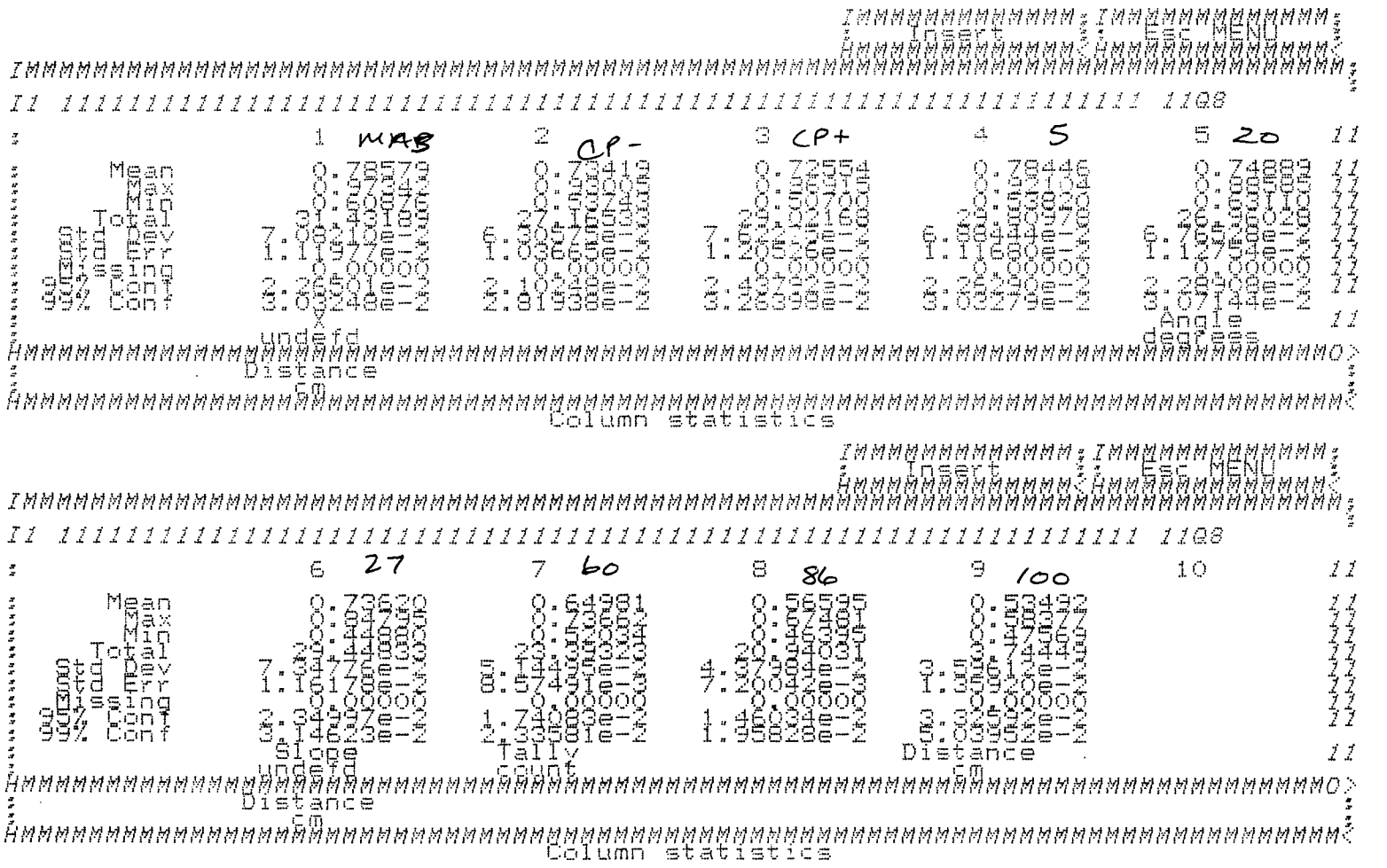
⇒ C2PBA





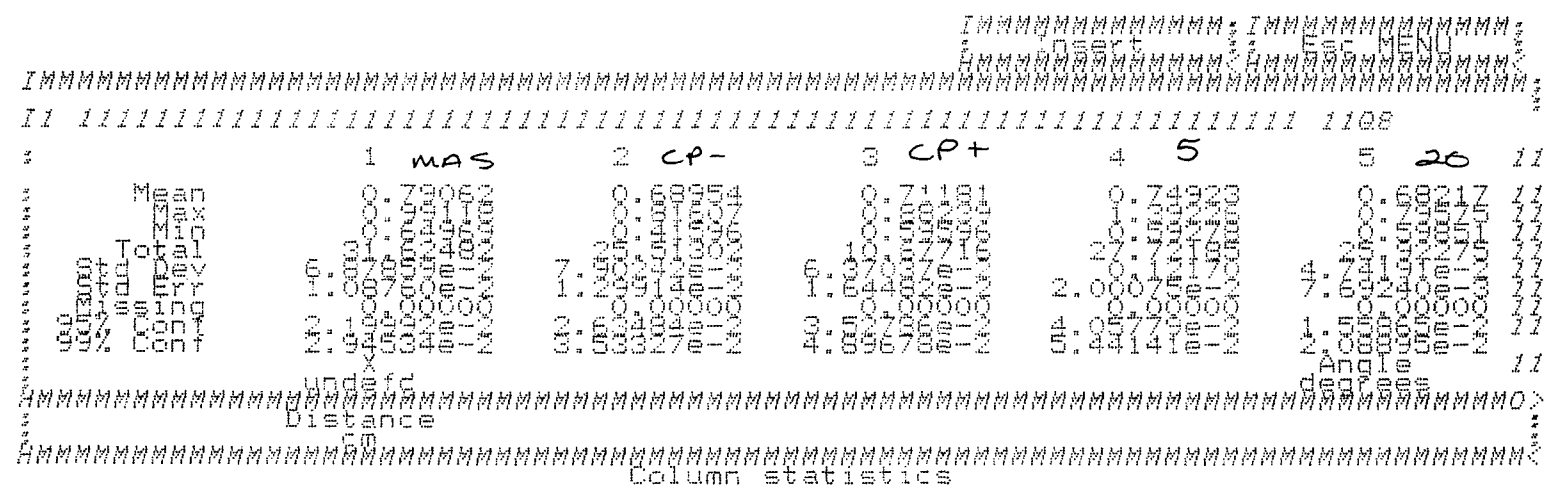
SBIR DEF. TEST
COUMARIN PB ACT

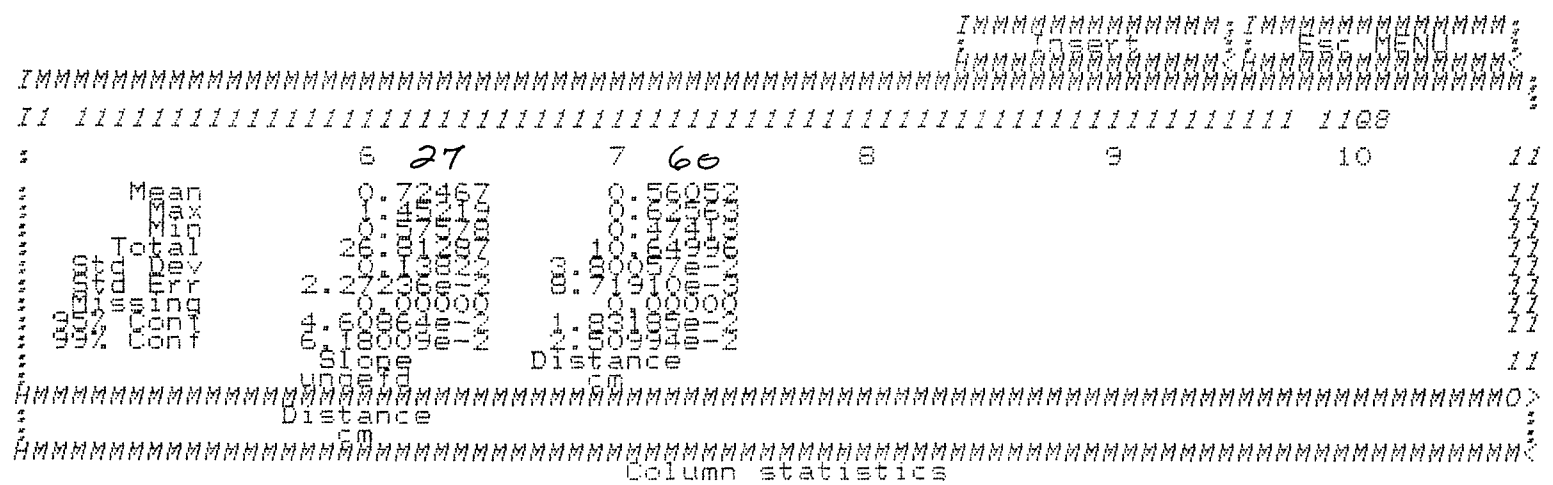
⇒ CPBA



SBIR DEF. TEST
COUMARIN ARO ACT

⇒ CAROA

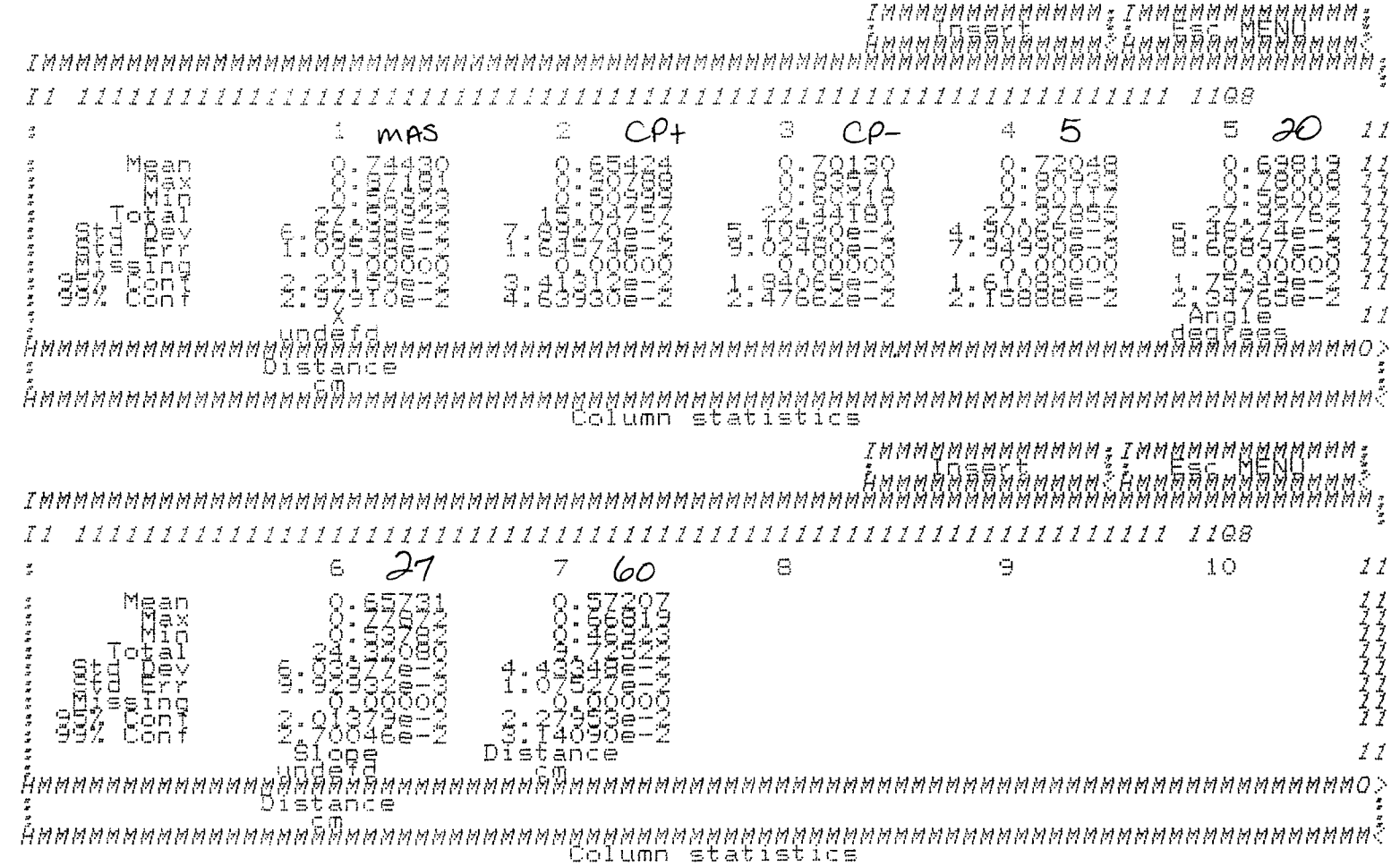




SBIR DEF TEST

COUMARIN MIX ACT.

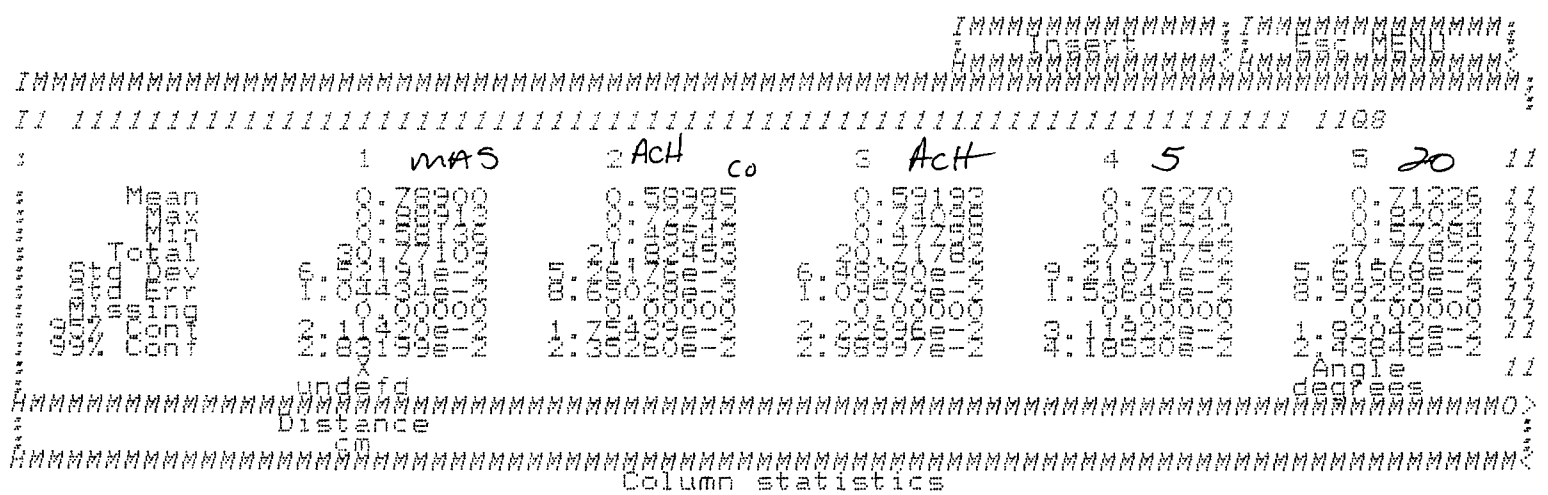
⟹ CMIXA

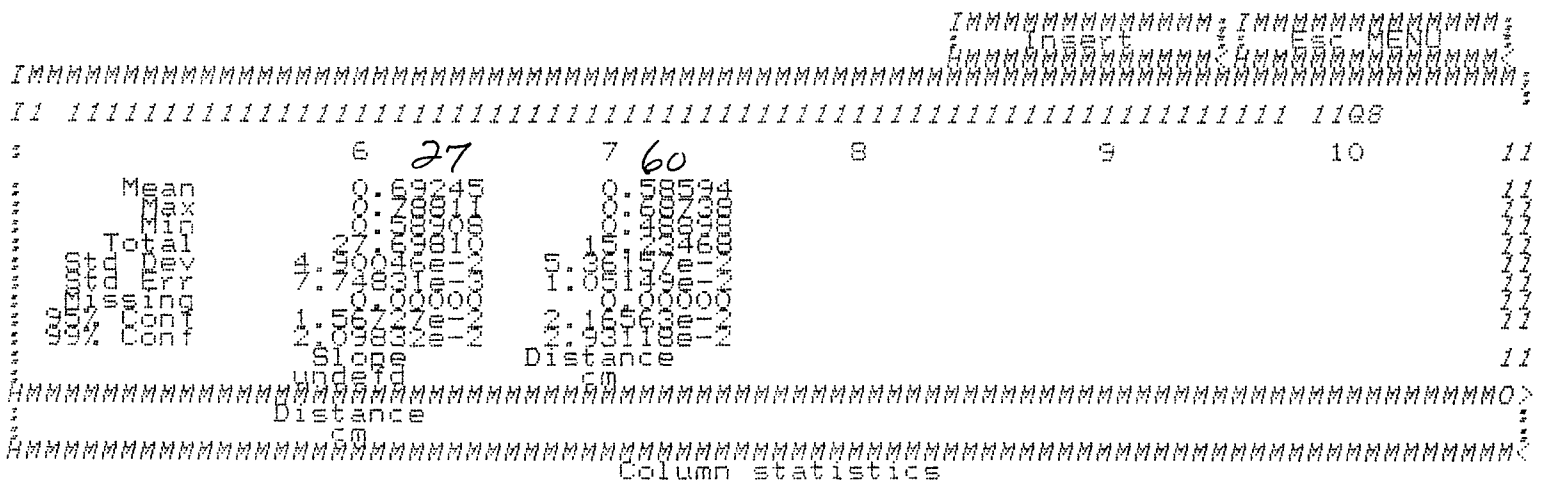


SBIR DEF. TEST

COUMARIN INH ACT

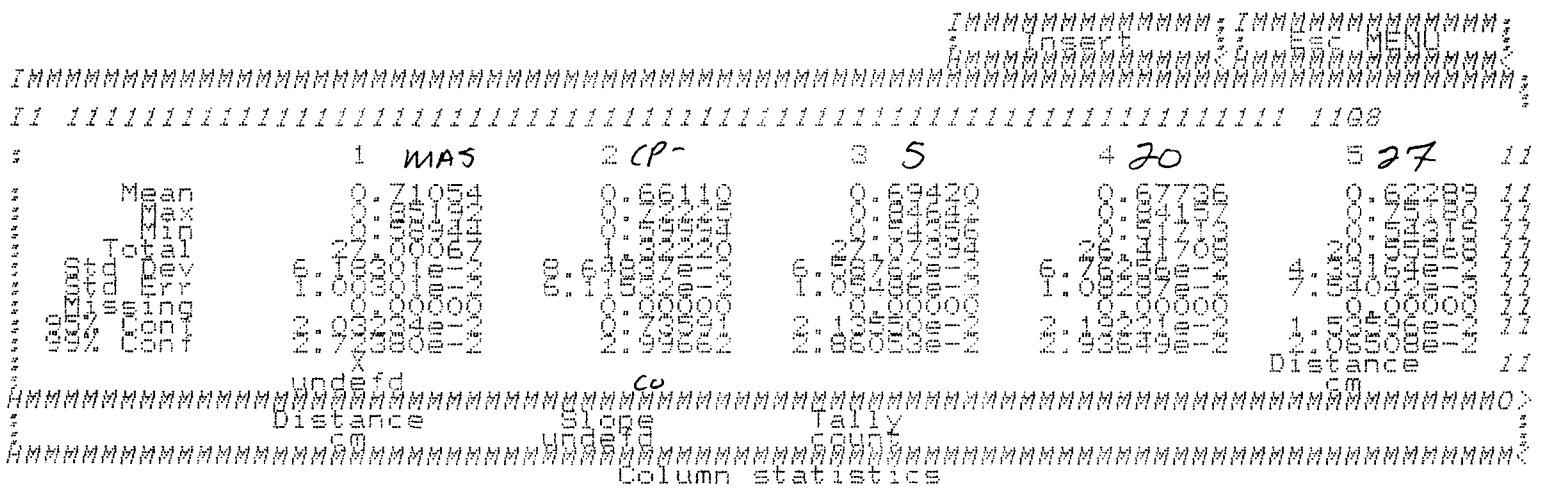
⇒ CINHA





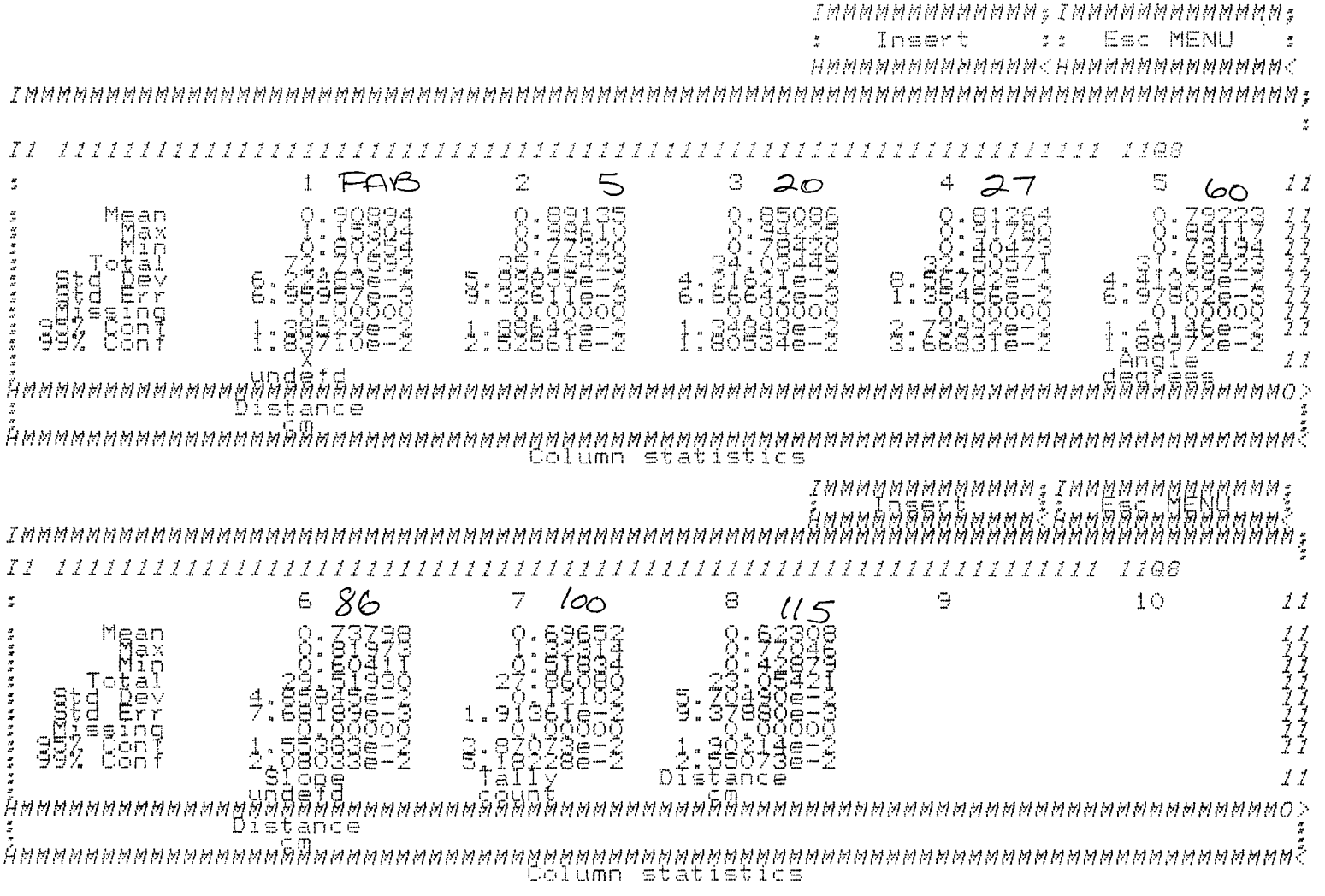
SBIR DEF. TEST
COUMARIN BNF ACT

⟹ CBNFA



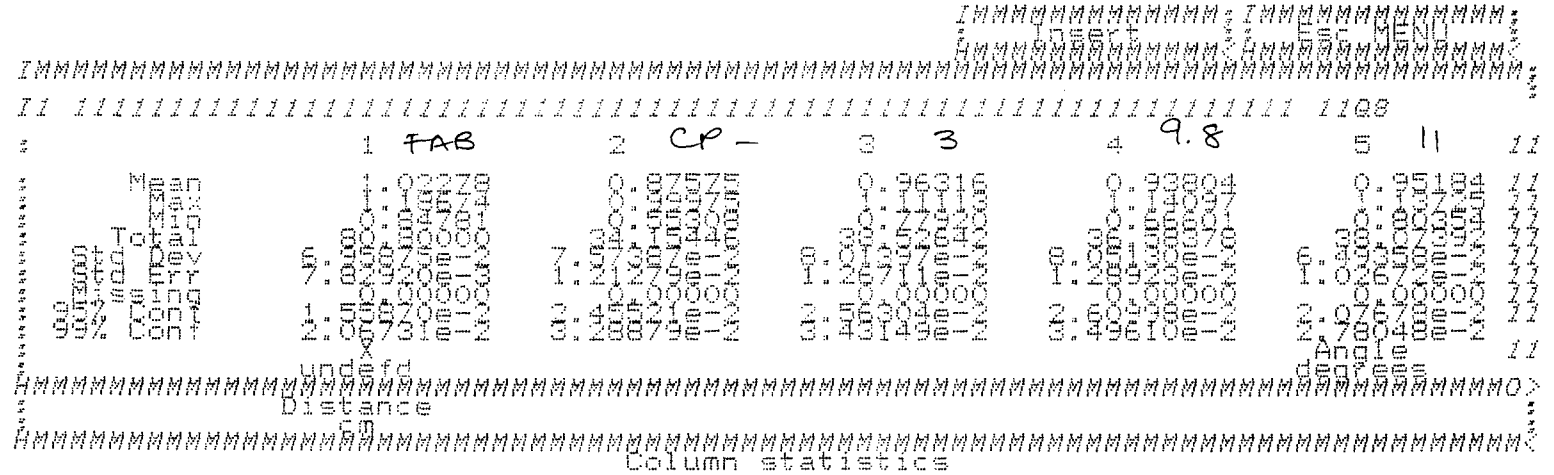
SBIR DEF.
COUMARIN UNACT.

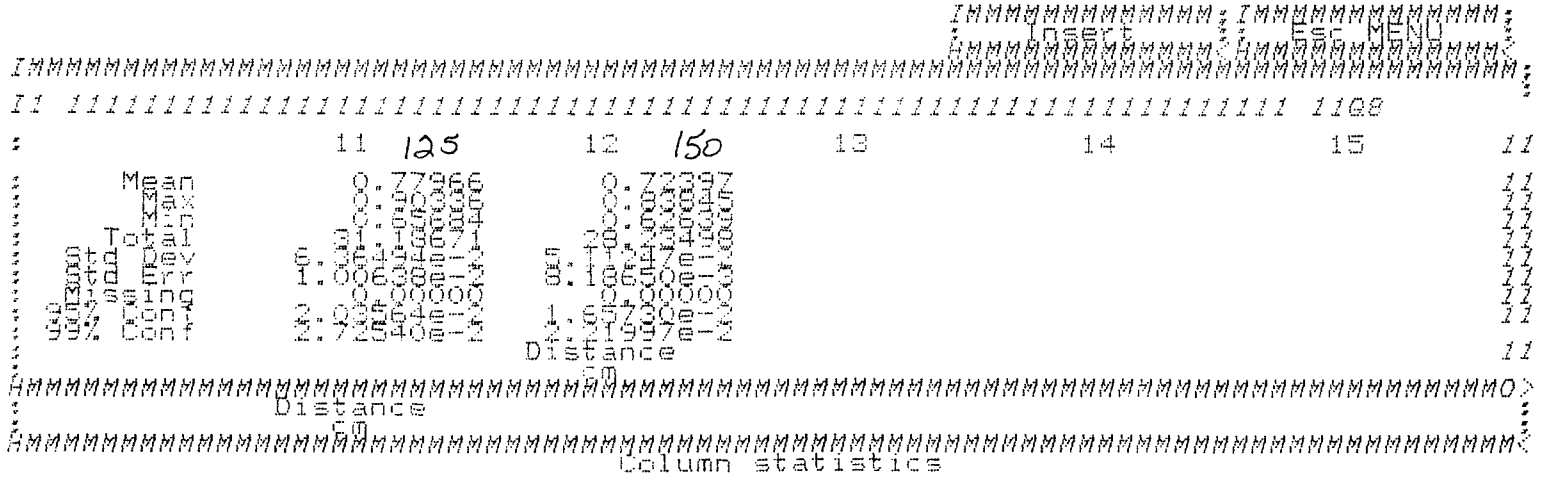
⇒ CUA



SBIR
2AAF MAI TEST
UNACT 1-97

⇒ 2AAFUA

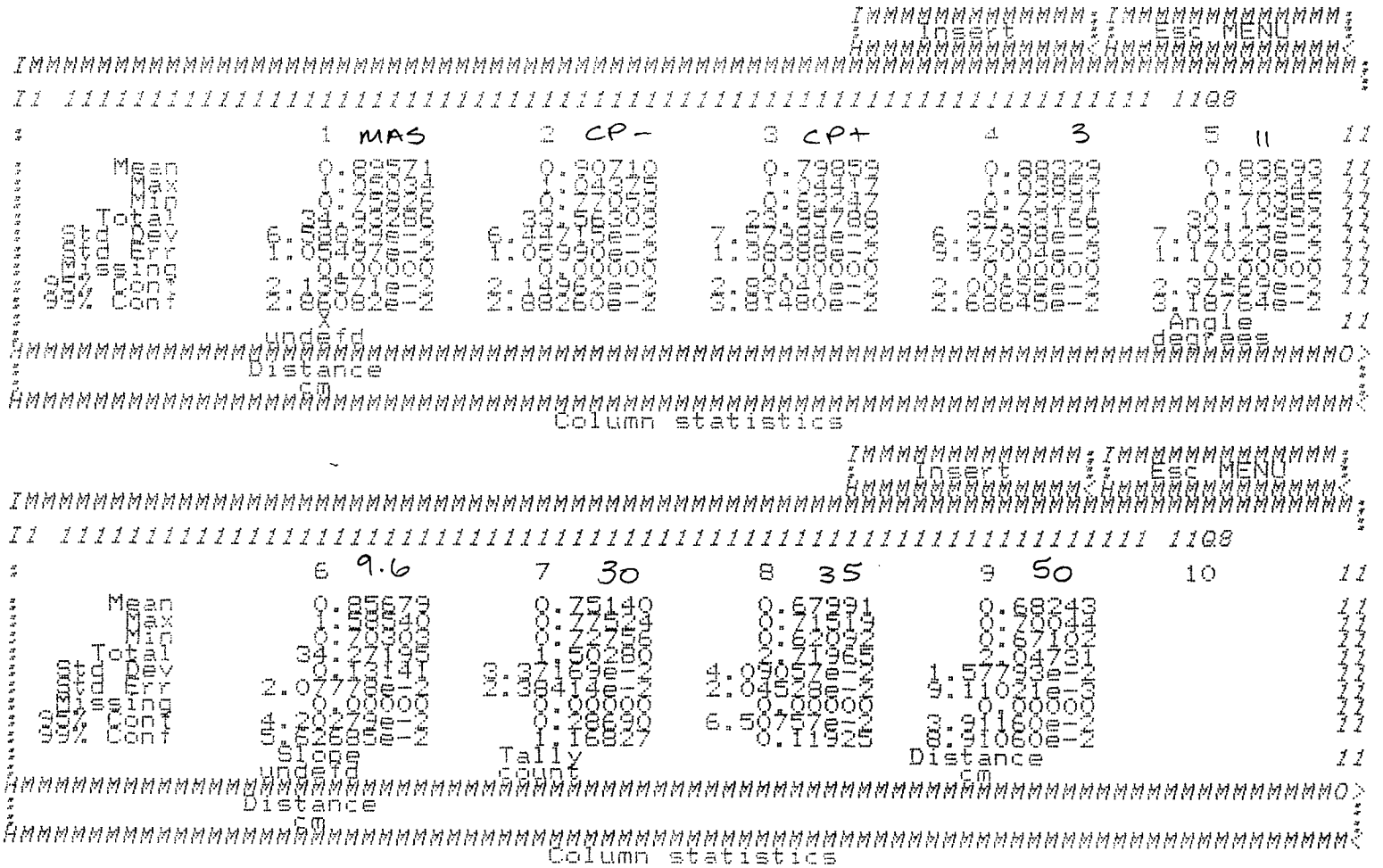




* 7 dry on tray unavailable to digitize

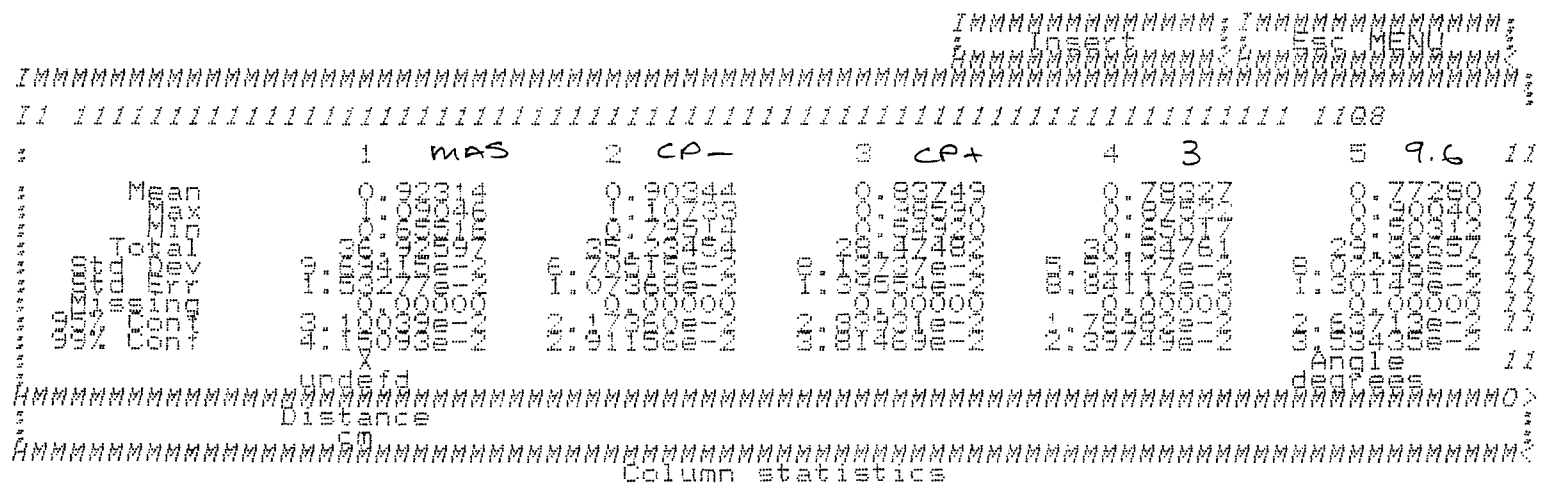
SBIR 2AAF MIX ACT. 1-97

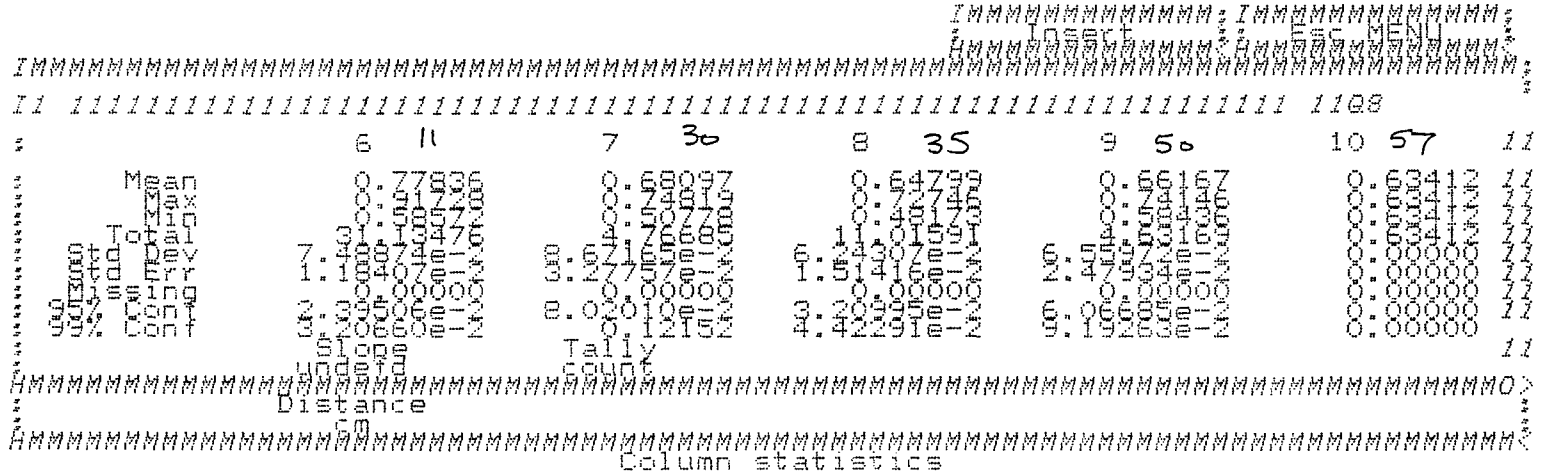
⇒ 2AAFMIXA

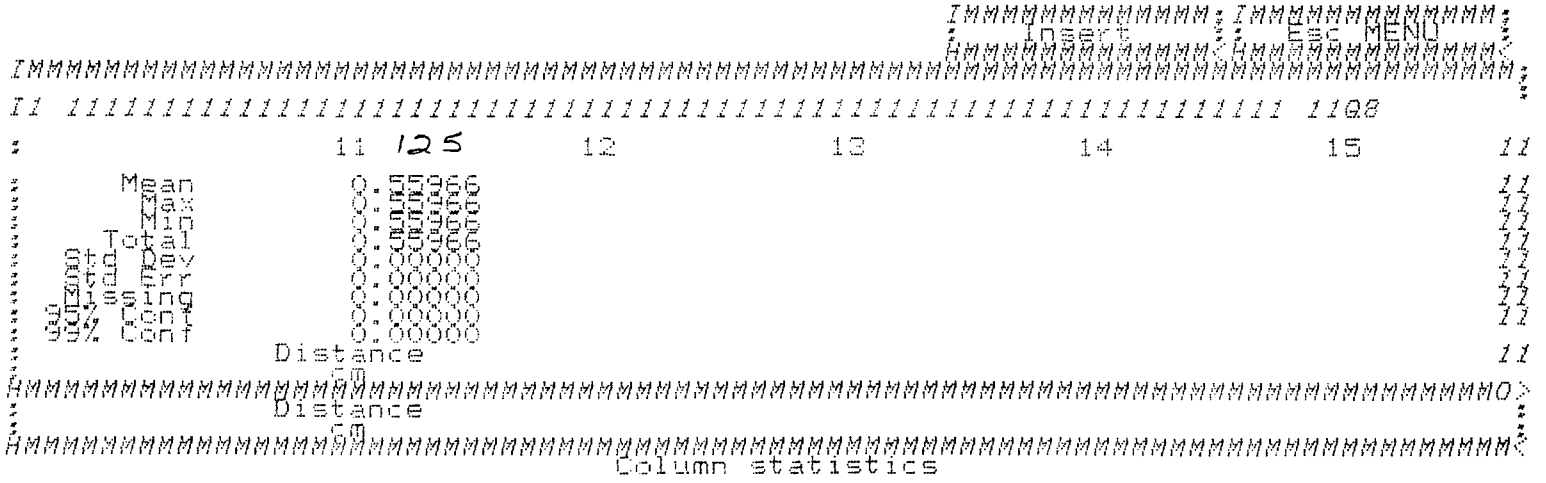


SBIR 2AAF
AROCHLOR ACT 1-97

⟹ 2AAFAROA



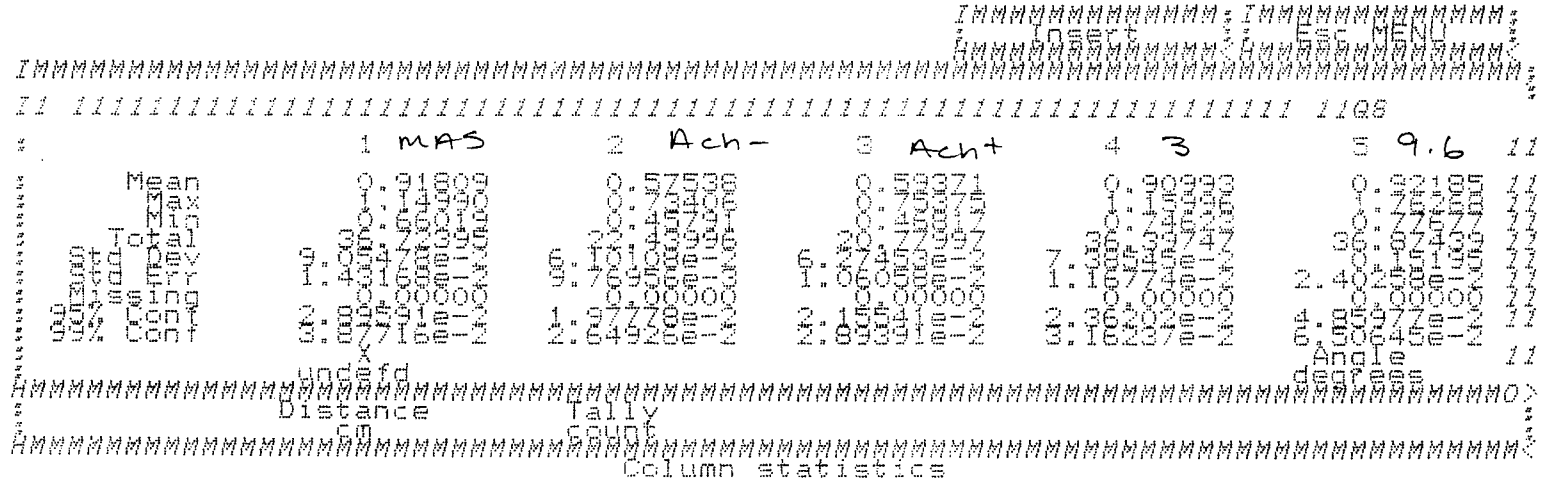


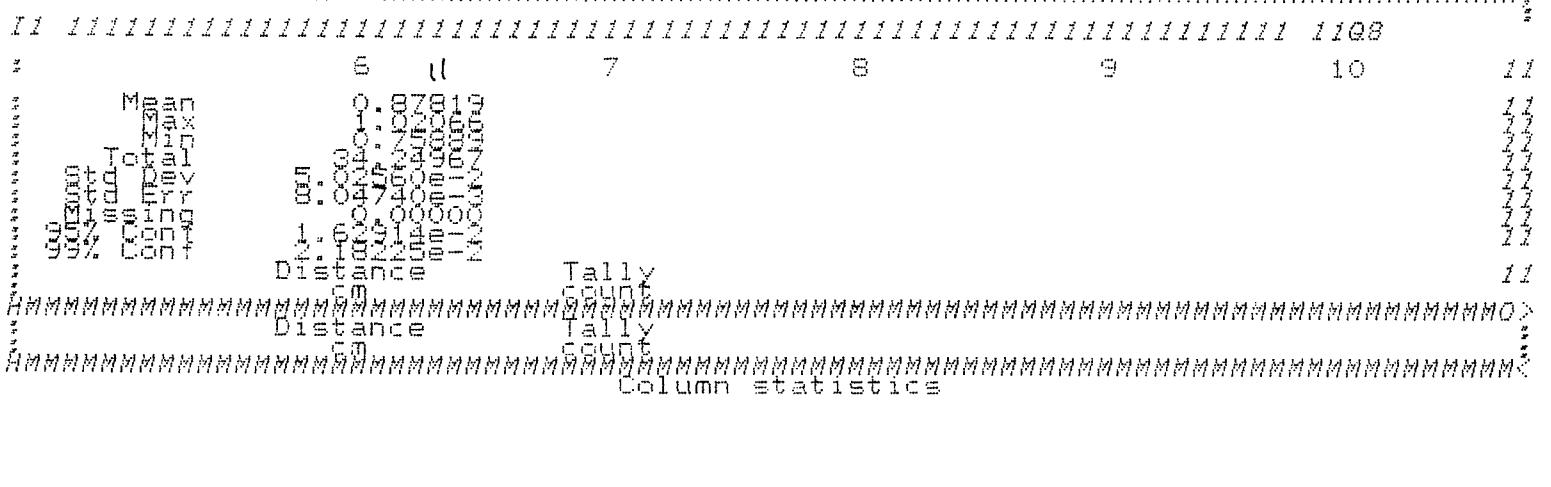


SBIR 2AAF

INH ACT 1-97

⇒ 2AAFINHA





FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		COUMARIN		
EC50=	59.8			
LC50=	168.7			
EC5	5.98			
EC16	19.136			
EC50	59.8			
EC84	100.464			
EC95	113.62			
LC5	16.87			
LC16	53.984			
LC50	168.7			
LC84	283.416			
LC95	320.53			

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CYCLOPHOSPHAMIDE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	5.50	6.00	6.50	7.00	7.50	8.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	2	13	23	39	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 6.76
VAR OF LN OF EST. : .74859D-04
95% LOWER CONFIDENCE: 6.65
95% UPPER CONFIDENCE: 6.88

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CYCLOPHOSPHAMIDE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	3.50	4.00	4.50	5.00	5.50	.00	.00	.	
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	1	6	22	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 5.83
VAR OF LN OF EST. : .11159D-03
95% LOWER CONFIDENCE: 5.71
95% UPPER CONFIDENCE: 5.95

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.05	.09	.10	.10	.13	.15	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	1	4	8	39	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: .11
VAR OF LN OF EST. : .16937D-03
95% LOWER CONFIDENCE: .11
95% UPPER CONFIDENCE: .11

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.02	.03	.03	.04	.04	.05	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	5	10	19	26	38	40	0	0	0

SPEARMAN-KARBER TRIM: 12.50%

SPEARMAN-KARBER ESTIMATES: EC50: .03
VAR OF LN OF EST. : .91693D-03
95% LOWER CONFIDENCE: .03
95% UPPER CONFIDENCE: .03

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.35	.40	.45	.50	.55	.05	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	3	7	13	40	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: .49
VAR OF LN OF EST. : .12753D-03
95% LOWER CONFIDENCE: .48
95% UPPER CONFIDENCE: .50

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	35.00	40.00	45.00	50.00	55.00	60.00	65.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	2	10	18	21	38	40	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 50.80
VAR OF LN OF EST. : .19949D-03
95% LOWER CONFIDENCE: 49.38
95% UPPER CONFIDENCE: 52.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	75.00	95.00	100.00	150.00	200.00	control 65.00		.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	1	6	11	40	38	40	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 168.74
VAR OF LN OF EST. : .77755D-03
95% LOWER CONFIDENCE: 154.17
95% UPPER CONFIDENCE: 184.16

Recalculated 11/13/96 DTF

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAC

RAW DATA:

CONCENTRATION(MG/L)	50.00	55.00	60.00	65.00	70.00	60.00	65.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	11	19	29	40	38	40	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 59.79
VAR OF LN OF EST. : .11453D-03
95% LOWER CONFIDENCE: 58.52
95% UPPER CONFIDENCE: 61.08

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		2-AAF		
EC50=	110			
LC50=	30			
EC5	11			
EC16	35.2			
EC50	110			
EC84	184.8			
EC95	209			
LC5	3			
LC16	9.6			
LC50	30			
LC84	50.4			
LC95	57			

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		COUMARIN		
EC50=	59.79			
LC50=	268.74			
EC5	5.979			
EC16	19.1328			
EC50	59.79			
EC84	100.4472			
EC95	113.601			
LC5	26.874			
LC16	85.9968			
LC50	268.74			
LC84	451.4832			
LC95	510.606			

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		TCE		
EC50=	490			
LC50=	51			
EC5	49			
EC16	156.8			
EC50	490			
EC84	823.2			
EC95	931			
LC5	5.1			
LC16	16.32			
LC50	51			
LC84	85.68			
LC95	96.9			

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		CYCLOPHOSPHAMIDE		
EC50=	4.83			
LC50=	6.76			
EC5	0.483			
EC16	1.5456			
EC50	4.83			
EC84	8.1144			
EC95	9.177			
LC5	0.676			
LC16	2.1632			
LC50	6.76			
LC84	11.3568			
LC95	12.844			

FETAX SUMMARY SHEET

Test Material: Cyclophosphamide	Test No.: 1
Source: Sigma SBR-Army	Investigator: Fort
CAS No. / Lot No.	Lab: SA
Composition/Purity	Test Start Date
Solvent / Conc.	Test End Date
	Test Units (i.e., mg/ml): mg/mL

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL: No. Dead or Malformed / Total Number X 100 = %	MORTALITY RECORD	MALFORMATION RECORD
	0 : 80 X 100 = 0 %	0 : 80 X 100 = 0 %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length ___ mm	Solvent Control Length ___ mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			

LC_{50}	6.76	EC_{50}	4.83
95% Confidence limits	6.65 - 6.88	95% Confidence Limits	4.71 - 4.95

TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50}) |

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	0 : 40 X 100 = 0 %	24 : 40 X 100 = ___ %
2500 mg/L	25 : 40 X 100 = ___ %	15 : 15 X 100 = 100 %

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	Cyclophosphamide
Date	11/12/96	**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							0 0	0	
FETAX							0 0	0	
6-AN 5.5							0 0	0	
6-AN 2500							12 13	25	
3.5							0 0	0	
4							0 0	0	
4.5							0 0	0	
5							0 0	0	
5.5							0 0	0	
6							0 0	0	
6.5						2 0	2 0	2	
7						8 5	8 5	13	
7.5						14 9	14 9	23	
8						20 19	20 19	39	
8.5						20 20	20 20	40	
						20 20	20 20	40	

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	Cyclophosphamide
Date	11/19/96	**Test Number**	

MALFORMATION	CONCENTRATION																									TOTAL
	F	F	6M 2.5	2500		3.5	4	4.5	5	5.5	6	6.5	7	7.5												
Severe			24	15																						
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	24	15		0	1	6	22	40	38	27	17	1												
Total No.	40	40	40	15		4	—	—	—	40	38	27	17	1												

Comments:

FETAX SUMMARY SHEET

Test No.	1

Test Material 2-AAF	Investigator Font
Source Sigma SBLR-Army	Lab SA
CAS No. Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/mL

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed / Total Number X 100 = %		
	0 : 80 X 100 = 0 %	0 : 80 X 100 = 0 %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			

LC_{50}	0.11	EC_{50}	0.03
95% Confidence limits	0.105 - 0.115	95% Confidence Limits	0.025 - 0.035

TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})	

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	0 : 40 X 100 = 0 %	18 : 40 X 100 = 45 %
2500 mg/L	25 : 40 X 100 = ___ %	15 : 15 X 100 = 100 %

FETAX MALFORMATION DATA

Investigator	For F	**Test Material**	2-AAF
Date	11/12/96	**Test Number**	1

MALFORMATION	CONCENTRATION																								TOTAL
	F	F	6 AF 5.5	b:AF 250		0.02	0.025	0.03	0.035	0.04	0.045	0.05	0.09	0.095	0.1	0.125	0.15								
Severe																									
Stunted																									
Gut																									
Edema																									
Multiple																									
Cardiac																									
Abdominal																									
Facial																									
Cephalic																									
Optic																									
Tail																									
Notochord																									
Fin																									
Face																									
Eye																									
Brain																									
Hemorrhage																									
Cardiac																									
Blisters																									
Other-specify																									
No. Malformed	0	0	18	15		5	10	19	26	38	40	40	39	36	32	1	—								
Total No.	40	40	40	15		40	40	40	40	40	40	40	39	36	32	1	—								

Comments:

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	2-AAF
Date	11/12/96	**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%	
FETAX							0	0	0		
FETAX							0	0	0		
5.5 6-AN							0	0	0		
2500 6-AN							15	10	25		
0.02							0	0	0		
0.025							0	0	0		
0.03							0	0	0		
0.035							0	0	0		
0.04							0	0	0		
0.045							0	0	0		
0.05							0	0	0		
0.09							0	1	1		
0.095							2	2	4		
0.1							5	3	8		
0.125							20	19	39		
0.15							20	20	40		

FETAX SUMMARY SHEET

Test No.	1

Test Material: TCE	Investigator: Fort
Source: Aldrich / Sigma	Lab: SA
CAS No. / Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent / Conc.	Test Units (i.e., mg/ml): mg/mL

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL: No. Dead or Malformed / Total Number X 100 = %	MORTALITY RECORD	MALFORMATION RECORD
	0 : 80 X 100 = 0 %	0 : 80 X 100 = 0 %
Solvent Control	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
Control Length ___ mm	Solvent Control Length ___ mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			

LC_{50} 0.49	EC_{50} 0.051
95% Confidence limits 0.48 - 0.50	95% Confidence Limits 0.049 - 0.052

TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50}) |

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %
2500 mg/L	___ : ___ X 100 = ___ %	___ : ___ X 100 = ___ %

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	TCE
Date	11/12/96	**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total		No	%
FETAX							0	0	0	
FETAX							0	0	0	
5.5 6-AN							0	0	0	
2500 6-AN							12	14	26	
0.035							0	0	0	
0.04							0	0	0	
0.045							0	0	0	
0.05							0	0	0	
0.055							0	0	0	
0.06							0	0	0	
0.065							0	0	0	
0.3							0	0	0	
0.35							0	0	0	
0.4							0	0	0	
0.45							2	1	3	
0.5							4	3	7	
0.55							8	5	13	
0.6							20	20	40	
							20	20	40	

FETAX MALFORMATION DATA

Investigator	FoF	**Test Material**	TCB
Date	11/12/96	**Test Number**	1

MALFORMATION	CONCENTRATION																									TOTAL
	F	F	5.5	2500		0.035	0.04	0.045	0.05	0.055	0.06	0.065	0.3	0.35	0.4	0.45	0.5	0.55	0.6							
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	16	14		0	2	10	18	21	38	40	40	40	31	33	27	—	—							
Total No.	40	40	40	14		40	40	40	40	40	40	40	40	40	37	33	27	—	—							

Comments:

FETAX SUMMARY SHEET

Test No.

Test Material Coumarin	Investigator Fort
Source Sigma SBIR-Army	Lab SA
CAS No. Lot No.	Test Start Date
Composition/Purity	Test End Date
Solvent Conc.	Test Units (i.e., mg/ml) mg/L

___ pH ___	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
$\frac{\text{No. Dead or Malformed}}{\text{Total Number}}$ X 100 = %	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%
Solvent Control	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			

LC_{50}	168.74	EC_{50}	59.79
95% Confidence limits	154.17 - 184.16	95% Confidence Limits	58.52 - 61.08

TEST TERATOGENIC INDEX (TI = LC_{50} / EC_{50})

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%
2500 mg/L	____ : ____ X 100 = ____%	____ : ____ X 100 = ____%

FETAX MORTALITY DATA

Investigator	Fort	**Test Material**	Coumarin
Date	11/12/96	**Test Number**	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX									
FETAX									
6-AN 5.5									
6-AN 2500									
0.045							0 0	0	
0.05									
0.055									
0.06									
0.065									
0.07									
0.075									
0.095							1 0	1	
0.1							3 3	6	
0.15							5 6	11	
0.2							20 20	40	
0.25							20 20	40	

FETAX MALFORMATION DATA

Investigator	Fort	**Test Material**	Coumarin
Date	11/12/96	**Test Number**	1

MALFORMATION	CONCENTRATION																											TOTAL
	F	F	5.5	250		0.045	0.05	0.055	0.06	0.065	0.07	0.075	0.09	0.1	0.15													
Severe																												
Stunted																												
Gut																												
Edema																												
Multiple																												
Cardiac																												
Abdominal																												
Facial																												
Cephalic																												
Optic																												
Tail																												
Notochord																												
Fin																												
Face																												
Eye																												
Brain																												
Hemorrhage																												
Cardiac																												
Blisters																												
Other-specify																												
No. Malformed	0	0	26	10		0	0	11	19	29	40	40	39	34	29													
Total No.	40	40	40	10		40	—	—	—	—	→	40	39	34	29													

Comments:

Appendix B

Metabolic Activation System Test Kit Design

Version 4
FETAX Microsome Kit

Contents:

☐ Aroclor 1254-induced ☐ Isoniazid-induced ☐ Phenobarbital-induced

☐ β- Naphthoflavone-induced ☐ Phenobarbital-β- Naphthoflavone-induced 1:1 mix

☐ Aroclor 1254-induced-Isoniazid-induced 1:1 mix

☐ Phenobarbital, β- Naphthoflavone-induced- Isoniazid-induced 1:1 mix

Lot #_____ Preparation date _____

When a mix is checked it means that a lot of microsomes from a rat induced with one substance is mixed with another lot of microsomes from a rat induced with another substance. The 1:1 means that the activities have been adjusted to the proper number of units of activity according to the Nash assay. Mixed microsomes offer the ability to cover a wider range of metabolic activity than a single broad spectrum inducer.

Microsome Specific Activity _____ Units/mg protein

Protein Concentration of Microsomes in Kit _____µg/ml
BioRad Protein Assay run with IgG standard

performed by _____date_____

Nash Units of microsomes in Kit _____U/ml

performed by _____date_____
FETAX
Positive Controls Date Performed_____ Technician _____

Microsome Quality Control:

FETAX positive control for Aroclor-induced microsomes.

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Cyclophosphamide		
4 mg/ml Cyclophosphamide + Microsomes		

Cyclophosphamide with Aroclor 1254-induced-microsomes should result in 50% mortality at the end of the 96 hrs.
Technician performing Assay _____Date:_____

FETAX positive control for Isoniazid-induced microsomes

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Acetyl hydrazide		
4 mg/ml Acetyl hydrazide + Microsomes		

Acetyl hydrazide with isoniazid-microsomes should result in 50% mortality at the end of the 96 hrs.

Technician performing Assay _____Date:_____

FETAX positive control for Aroclor-1254 induced and Isoniazid-induced (mixed) microsomes or Phenobarbital, β- Naphthoflavone-induced- Isoniazid-induced 1:1 mix

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Cyclophosphamide		
4 mg/ml Cyclophosphamide + Microsomes		

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Acetyl hydrazide		
4 mg/ml Acetyl hydrazide + Microsomes		

Cyclophosphamide with Aroclor 1254-induced-microsomes should result in 50% mortality at the end of the 96 hrs.

Acetyl hydrazide with isoniazid-microsomes should result in 50% mortality at the end of the 96 hrs.

Technician performing Assay _____Date:_____

Storage:

Box 1-Microsomes: These must be stored at -80°C. If such a freezer is not available remove the tubes from the box and freeze under liquid nitrogen. There is one tube for each day of the experiment. Discard any left over microsomes and do not refreeze.

Box 2-Generator, glucose 6-phosphate dehydrogenase, cyclophosphamide (Aroclor 1254 positive control) and acetyl hydrazide (isoniazid positive control). Store frozen at -20°C.

Kit Design:

Each kit is designed to allow the contents to be directly pipeted into a 50 ml Erlenmeyer flask without further calculation. The total volume in the flask is 20 ml regardless whether glass or plastic dishes are being used. Generally, plastic dishes are used to help maintain sterility. However, autoclaved glass dishes may be used when the test material binds to plastic. Simply discard the remainder and thaw the contents of new tubes on each successive day of the test.

Thawing instructions:

Bring tubes to room temperature and with gentle rocking motion of the wrist mix the contents of the tube. Freezing often causes a salting out effect thereby requiring that the tube contents be resuspended in solution.. As soon as the last ice melts, place the tube in an ice bucket. Never shake or vortex a tube to mix contents, always stir or gently rock into solution. This is especially true for the microsomes. Frothing indicates denaturation of protein which means enzymatic function is lost.

Adding contents to the 50 ml Flask: Use an appropriate Pipetman for small quantities and use a disposable plastic Pipet for larger volumes.

1. If appropriate, add correct amount of cyclophosphamide to flask or acetyl hydrazide.
2. If appropriate, add test compound.
3. Calculate the amount of additional FETAX-AB needed to reach 20 ml. Add this amount and gently swirl to mix.
4. Add correct amount of Generator to flask.
5. Add correct amount of microsomal protein to flask.
6. Add correct amount of Glucose-6-Phosphate Dehydrogenase to flask.
7. The add 10 ml to each glass dish or 8 ml to each plastic dish as needed. Discard any remaining solution or use it for chemical analysis.

Kit Contents:

Compo-nent	Amount to Add/day	day 0	day 1	day 2	day 3	Kit
	(µl)	Total Quantity (ml)	Total Quantity (ml)	Total Quantity (ml)	Total Quantity (ml)	Grand Total (ml)
Generator	192.5	6.3	6.3	6.3	6.3	25.2
Glucose-6-Phosphate dehydro-genase	62	2	2	2	2	8
Micro-somal Protein	191	6.3	6.3	6.3	6.3	25.2
Cyclophos-phamide	10,000	21	21	21	21	84
Acetyl hydrazide						

* Each kit holds enough material for 32 Petri dishes that contain MAS. There will always be some material left over.

Kit Supplies Materials to Prepare the Following Dishes:

Dishes in an experiment that do not contain MAS.

FETAX solution only - 4 dishes

FETAX-AB solution - 2 dishes

FETAX solution plus 4 mg/ml cyclophosphamide (part of positive control)-2 dishes.

Dishes in an experiment that contains MAS.

FETAX- generator- microsomal protein (negative control)- 2 dishes.

FETAX- generator- microsomal protein treated with carbon monoxide-dithionite- 4 mg/ml cyclophosphamide (negative control)- 2 dishes.

FETAX- generator- microsomal protein + LC50 of test compound (control for interaction between MAS and test compound)- 2 dishes.

FETAX- generator- microsomal protein + EC50 of test compound (control for interaction between MAS and test compound)- 2 dishes.

FETAX- 4 mg/ml cyclophosphamide- generator- microsomal protein (part of positive control for Aroclor-induced microsomes)- 2 dishes.

FETAX- generator- (negative control for generator- no microsomal)- 2 dishes.

For different concentrations of the test material:

FETAX-generator-microsomal protein-test compound-2 dishes per test concentration (20 total).

Total dishes requiring MAS-32.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB218773
ADB223531
ADB230017
ADB223528
ADB231930
ADB226038
ADB224296
ADB228898
ADB216077
ADB218568
ADB216713
ADB216627
ADB215717
ADB218709
ADB216942
ADB216071
ADB215736
ADB216715
ADB215485
ADB215487
ADB220304
ADB215719
ADB216072
ADB222892
ADB215914
ADB222994
ADB216066
ADB217309
ADB216726
ADB216947
ADB227451
ADB229334
ADB228982
ADB227216
ADB224877
ADB224876
ADB227768
ADB228161
ADB229442
ADB230946
ADB230047
ADB225895
ADB229467
ADB224342
ADB230950
ADB227185
ADB231856
ADB229914
ADB229497
ADB230947
ADB282209
ADB270846
ADB282266
ADB262442
ADB256670